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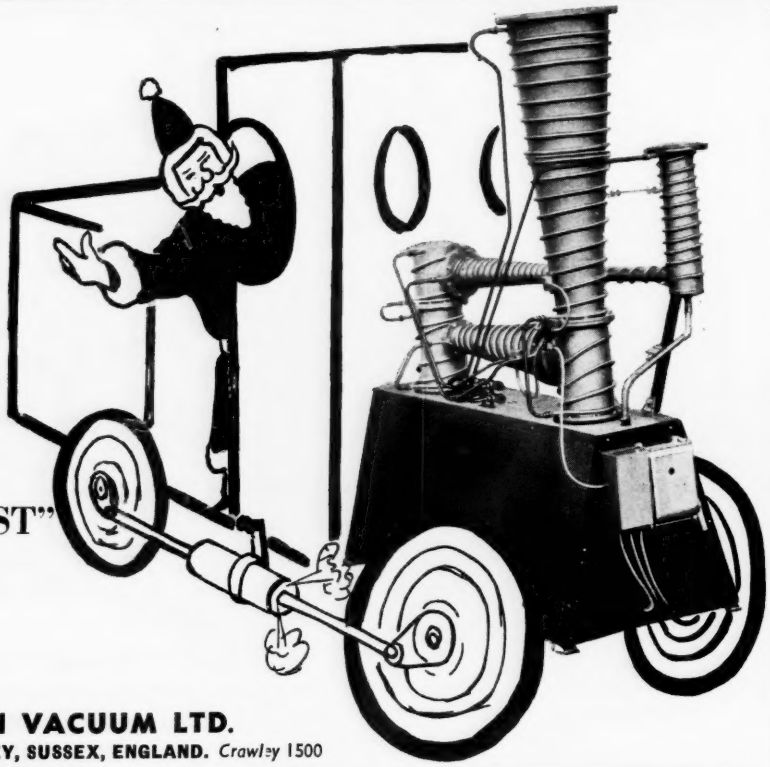

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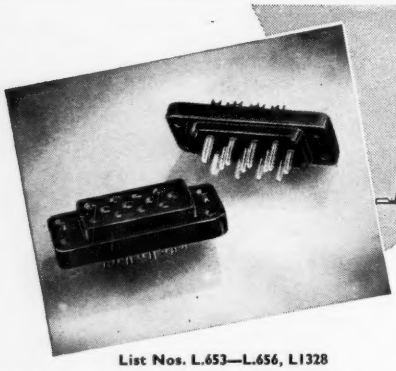

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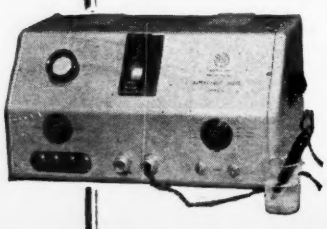

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SCIENTIFIC RESEARCH IN GREAT BRITAIN

THE tenth annual report of the Advisory Council on Scientific Policy*, covering the year 1958–59, deals with three main topics: scientific man-power, space research and overseas scientific relations. Of the first, it may be said that the Advisory Council comments briefly on the results of a further survey of scientific and engineering man-power made last winter by the Ministry of Labour and National Service along the same lines as that conducted in 1956. The Council agrees with the Scientific Man-power Committee that the experience of three years is an insufficient basis for a detailed re-assessment of the long-term demand, but its general comments are more conveniently discussed in considering this second report on Scientific and Engineering Man-power in Great Britain itself, which has been published separately.

Besides these surveys, the main report refers briefly to the development of the National Lending Library of Science and Technology, which is now expected to be in full operation by 1962, and to the inquiries made during the year into research contracts of United States Agencies with British universities and institutions in an endeavour to assess the total amount of such financial support and the principles on which it is given. On the former matter, it is startling to read that the Advisory Council believes that its responsibilities towards such schemes can now be regarded as satisfactorily concluded. The report describes briefly what is being done in the way of buildings and the collection of scientific literature by the Lending Library Unit of the Department of Scientific and Industrial Research, and refers appreciatively to the sponsorship by this Lending Library Unit of translations of eight Russian periodicals.

It is only two years since the Advisory Council first reported progress towards the establishment of a National Lending Library of Science and Technology, and even a year ago it was expressing concern at the failure to establish the National Reference Library of Science and Invention. The two projects are inter-related and serious doubts are entertained by competent judges as to how far either is likely to be efficient in the absence of the other, apart from any question of duplicating resources. For that reason and for the further reason that the Advisory Council's own recommendations for the National Lending Library included collaboration with the existing regional and specialist library services in the supply of scientific literature, this statement in the report reads rather like dereliction of duty. Even with the appointment of a consultative committee to advise the Department of Scientific and Industrial Research from the point of view of the users of the Library on the services to be provided by it, the Advisory Council ought not to disclaim either responsibility or interest.

The Advisory Council's report does not mention the appointment of this committee, the membership of which has been announced, but, on the Advisory Council's own showing, an adequate lending and reference service of scientific libraries bears so closely on both the supply of scientific and technical man-power and its efficient use that the Advisory Council can scarcely avoid a continuing interest in the provision of such services. Incidentally, the users do not seem to be adequately represented on that committee. In any event, it will probably be necessary for the Council to continue to exert its influence in order to overcome the obstacles to co-operation and to ensure that adequate resources are placed at the service of the library system as a whole. Indeed, from this point of view, although the Roberts Report on the Public Library Service was addressed to the Minister of Education, some comment on the report might have been looked for from the Advisory Council, for Sir Sydney Roberts's Committee gave due consideration to the relation of public libraries to other libraries, including scientific libraries.

On the matter of research contracts with British universities and institutions, the Advisory Council estimates that financial support for research in Britain from Government agencies and charitable foundations in the United States amounts to about £1·5 million per annum, of which about 1·5 million dollars comes from charitable foundations, such as the Rockefeller, Ford and Kellogg Foundations, a like sum from United States civilian agencies such as the National Science Foundation, and about half that sum from United States Service departments and agencies; and most of the money from Government agencies has so far been in the form of contracts for specific projects. While welcoming the substantial support this affords for research in Britain, the Advisory Council recognizes that research grants, and particularly specific contracts of this magnitude, could influence considerably the general pattern of research. Accordingly, with the full co-operation of the United States authorities, who fully appreciate the position, it is seeking to extend to all such contracts existing arrangements by which all research contracts placed by United States Service departments are notified to the British departments and research councils concerned. Without interfering with the freedom of university departments to accept or reject contracts which are offered to them, the arrangements should make it possible to co-ordinate British and United States offers of financial support for research, and should also ensure that the general terms of the various contracts are on a uniform basis. While the Advisory Council hopes that in future a substantial proportion of the funds available will be provided in the form of grants, its inquiries did not indicate that the terms of contracts and security considerations hindered either the freedom of the research worker concerned or the publication of his results.

* Annual Report of the Advisory Council on Scientific Policy 1958–1959. (Cmnd. 893.) Pp. iv+14. (London: H.M. Stationery Office, 1959.) 1s. net.

The linking of research and other scientific activities in the United Kingdom is further illustrated in the section of the Advisory Council's report which deals with overseas scientific relations. Reference was made in the Advisory Council's last annual report to the establishment by the North Atlantic Council of a Science Committee on which the United Kingdom representative is Sir Solly Zuckerman. This Committee has recently set up a small study group of scientists, under the chairmanship of M. Louis Armand, to review the whole field of scientific effort in the North Atlantic Treaty Organization countries, and to direct attention to underlying weaknesses or gaps, and the results of this study are expected to be available next year. Under the Organization for European Economic Co-operation, which is at present reviewing its committee system, the Committee on Scientific and Technical Personnel has developed a senior visiting fellowhip programme, designed to enable research institutions to send their own research workers abroad, or to invite research workers from foreign institutions to visit them; this appears to be meeting a need in many countries. The Committee on Applied Research also has developed a number of practical co-operative research projects.

The Government's decision to set up an Overseas Research Council is warmly welcomed in the Advisory Council's report, which stresses the urgency and importance of the matters with which the new Council will have to deal. Emphasizing the value of the contribution which the United Kingdom has to offer to the development of the overseas territories, the Advisory Council hopes that the new Council will both be able to advise Ministers on the type of assistance that is most needed and also act as a focus of interest in the application of research, both in and for the overseas territories. The new Council should, it thinks, prove a useful complement in the scientific field to the work being undertaken in the educational field as a result of the recent Commonwealth Education Conference.

The close relation between research and education in the overseas territories is one reason for hoping that, while the detailed activities in research overseas will doubtless in future be described in the annual reports of the Overseas Research Council, the Advisory Council on Scientific Policy will not cease to concern itself with the broad issues and with the inter-relations with research in other fields and with education. Moreover, it has still to be seen if the Overseas Research Council can supply that overall conspectus of overseas research rather than the sectional reports, however admirable in themselves, to which "Colonial Research" has hitherto been limited. Such a conspectus is increasingly desirable in view of the growth in scale and extent of research in the Colonial and other overseas territories, and of the collaboration with the United States in this field. The appointment by the Lord President of the Council, pending the establishment of the Overseas Research Council, of a scientific panel, under the chairmanship of Sir Alexander Todd, to collaborate with a corresponding American panel, set up by the National Science Foundation in the United States, to prepare a report on the application of science to problems in Africa for the United States International Co-operation Administration is a step in the right direction.

It was in accordance with the advice of the Advisory Council on Scientific Policy that the British Government authorized a programme for the design and construction of instruments to be carried in a series of Earth satellites, and with the establishment of a Steering Group under Sir Edward Bullard to supervise and co-ordinate the general Government interest in the programme, so that the direct concern of the Advisory Council with the present programme of space research terminated. Nevertheless, the Advisory Council, in the present report, does not content itself merely with summarizing concisely the steps which led to the initiation of that programme, and the subsequent agreement of the United States National Aeronautics and Space Administration to provide space in a series of satellites to be launched over a period of three to four years for a number of British instruments designed to carry out a scientific programme under the general supervision of the British National Committee for Space Research. It also takes the opportunity of setting out principles which it believes should underlie British participation in this field of endeavour.

Space research, it is pointed out, especially when it proceeds beyond the stage of launching small Earth satellites, must make enormous and increasing demands on scientific and financial resources, and we in Great Britain cannot hope to provide all the resources necessary to develop space exploration independently on an equal footing with the United States and the U.S.S.R. Nevertheless, the Advisory Council is convinced that British scientists have an important and useful part to play in this new field, and that if they are provided with the means to carry their scientific instruments into space, carefully designed experiments with Earth satellites can contribute to the solution of important scientific problems and often provide the most economical method of obtaining the data needed. Ventures into space with lunar, planetary or solar probes would be far more costly, and in the Advisory Council's opinion the scientific and technological return is unlikely to be commensurate with the cost. British participation in such activities at the present time is not recommended.

The Advisory Council does not expect the scientific programme at present authorized to have in itself any adverse effect on the man-power situation in British industry; indeed, there may be some stimulus to micro-instrumentation. Nevertheless, the Advisory Council reiterates that the technological advantages likely to accrue from the development of a British rocket and satellite programme designed for civil purposes, over and above the effects of the existing military missile programme, would be insufficient to justify the very considerable expenditure involved. When the results of the design studies at present being carried out on the adaptation of British military

rockets are available, it will be possible to decide whether the additional expenditure and diversion of effort involved in a purely British satellite programme may be justified by the results which can be expected.

The Advisory Council is not unmindful of the claims of prestige, but rightly it stresses the all-important question of priorities for a nation with limited resources. Once again it emphasizes the importance of adequate expenditure on research and development in those fields which vitally affect the welfare of our people, the strengthening of our economic position and the improvement of standards of living in the under-developed countries, particularly in Commonwealth countries and Colonial territories. It is incontestably right in urging that it would be the grossest folly to leave these needs unsatisfied in order to embark on a large programme of space exploration on a purely national basis, and in suggesting that in such a field the right approach is that of international collaboration.

There may well be other fields in which the contribution appropriate to the special skills and resources available in Great Britain is best made on an international basis, and the newly established co-operative agreement with the United States already noted, and the further agreement for United Kingdom participation in a joint United States/Canadian Working Party to conduct experiments on the ionosphere by means of rockets are to be welcomed on general as well as on specific grounds. The Advisory Council's latest report provides conclusive evidence of the value of the Council, provided that its authority is properly sustained and its recommendations are effectively presented. It is manifest that Lord Hailsham, as Minister for Science, has at his hand an admirable instrument, with a realistic sense of priorities and the wide vision and clear understanding that are essential in securing co-operation and co-ordination in either science or technology.

A HISTORY OF MAGIC AND EXPERIMENTAL SCIENCE

A History of Magic and Experimental Science
By Prof. Lynn Thorndike. Vols. 7 and 8: The Seventeenth Century. Vol. 7: Pp. x+695. Vol. 8: Pp. viii+808. (New York: Columbia University Press; London: Oxford University Press, 1958.) 80*s*. net each volume.

THE publication by Prof. Lynn Thorndike of Vols. 7 and 8 of his great work, "A History of Magic and Experimental Science", completes a task on which he has been engaged for more than fifty years. The first two volumes appeared in 1923[1], but the author's interest in the subject extends back to a much earlier stage in his career. While still a student at Columbia University, New York, in 1902–3, it was suggested to him that a study of magic in medieval universities would form a suitable subject for a master's degree. The more immediate outcome of his initial researches in this field appeared in 1905, in a book entitled "The Place of Magic in the Intellectual History of Europe. This was followed by his "History of Medieval Europe".

After leaving Columbia, Prof. Thorndike served as associate professor of history in the Western Reserve University, and while there the first two volumes of his *magnum opus* were published. Shortly afterwards, in 1924, he returned to Columbia on appointment as professor of history in that University. It is evident that by this time Prof. Thorndike had already acquired a deep interest in the history of science, for he was one of the founder members of the History of Science Society established in the United States in 1924, later serving as its president; and in 1928 he was instrumental, with others, in setting up the Académie internationale d'Histoire des Sciences. It is from the fusion of these two interests, medieval history and the history of science, that his eight-volume work has grown.

The first two volumes cover the first thirteen centuries of the Christian era. They were reviewed by George Sarton, in *Isis*[2], and the review was a critical one. While admitting that the work was one of major importance, the reviewer expressed the opinion that the title was so misleading as to be deceptive. In Sarton's view, it was not a history of the science of the period, still less of experimental science, as these two terms should be understood. While there is something to be said for Sarton's point of view, it seemed a little pedantic, because, as he later admitted, what passed for science in medieval times was, for the most part, inseparably linked with the practice of astrology, alchemy and divination, and with the lore of gems and amulets.

Volumes 3 and 4 were published in 1934, and cover the fourteenth and fifteenth centuries. In Vol. 3 (p. 22) Prof. Thorndike met Sarton's criticism, intentionally or otherwise, with the statement that his work was to be regarded as a history of the relations between magic and experimental science, and this certainly defines the scope of the work rather more closely. These two volumes also were reviewed by Sarton[3], and this time, while repeating his opinion that the title was misleading, he was less critical; and he conceded that, since medieval thought was governed by astrology, alchemy and divination, Prof. Thorndike's work might be called a history of scientific thought. He pointed out, not unreasonably, that the term 'experimental science' was used by the author in far too loose a sense.

Vols. 5 and 6, which appeared in 1941, cover the sixteenth century; and, in Vol. 5 Prof. Thorndike, in discussing the first six volumes, states: "Like Moses we have brought the reader through the wilderness to within sight of the promised land of modern science. It remains to be seen whether we shall enter in or whether we shall content ourselves with viewing the prospect o'er from the other—magical—side of Jordan". Fortunately, the author decided to cross this intellectual Jordan, and continues his story to the end of the seventeenth century in his last two volumes.

Prof. Thorndike has deliberately restricted his study, in time, to the Christian era, and in space to the Western world. In these last two volumes he has moved forward to the seventeenth century, to the period when the great expansion in the search for knowledge took place, which has been called by Dr. Charles Singer the 'insurgent' century and by others one of scientific revolution. It is in this period that many of the experimental and observational sciences have their main roots and begin to take on a more modern form, and for this reason Vols. 7 and 8 are likely to have a wider appeal than the earlier ones. Their main thesis, however, is the extent to

which the belief in magic and the occult, universal in preceding centuries, lingered on through the seventeenth century, even in the minds of learned men, a fact that is not usually emphasized in the more conventional histories of science, though the evidence is ample. These last two volumes provide a link between the age of magic and that of modern science.

The two volumes cover 1,500 pages, and it is impossible to discuss their contents in detail within the limits of a review, but the following selection from the titles of the forty chapters gives an indication of the field covered. Vol. 7 includes the following: "Backward Glance"; "Kepler and Galileo"; "Francis Bacon"; "Alchemy and Iatro-Chemistry to 1650"; "Mineralogy"; "Natural Magic"; "Interest in the Occult in German Universities"; "Harvey and Patin"; "Descartes"; "The Underground World of Kircher and Becher"; and "Artificial Magic and Technology". Vol. 8 has chapters on "Natural History, especially of Animals"; "Botany"; "Chemical Courses and Manuals"; "Robert Boyle"; "Academies and Scientific Societies"; "Astrology after 1650"; "Medicine and Physiology"; "Illicit Magic"; "Sir Isaac Newton"; and "The Last Decade".

Prof. Thorndike's treatment of the scientists and subjects discussed always emphasizes the credulity of the age. His opinions are personal views, uninfluenced by the generalizations of other writers, and based solely on his own very extensive reading. For example, of Robert Boyle he writes: "In Boyle himself we have an example *par excellence* of one beginning to shuffle off the mystic robes of the magician and putting on the habiliments of modern science"; but he considers the statement by a recent biographer of Boyle that the latter is generally known to have been an alchemist, "accepting theoretically and practically the doctrine of the transmutation of the elements"[4] is too strong an estimate of Boyle's position. Some chapter headings might lead the unsuspecting reader to anticipate a discussion of the beginnings of systematic knowledge of some particular science, but he is likely to be disappointed. That on mineralogy, for example, is almost solely concerned with the belief in the supposedly occult or medicinal properties of gems and minerals.

Prof. Thorndike's work is in no sense a systematic or even a complete history of experimental science, or of science in general. It is a very detailed and fully documented account of beliefs that held sway for centuries before the scientific revolution, beliefs that were so deeply ingrained in the human mind that they were only with difficulty discarded by the scientists and naturalists who actually brought about this revolution. It is a work of great erudition. The author records that in the last two volumes some 1,600 authors and men of learning are mentioned. To the majority of readers most of these names are likely to be unfamiliar, if not entirely unknown. If known, the books they wrote (some exist only in manuscript form) are frequently of such rarity as to be difficult of access to the average reader; and, furthermore, the fact that many are written in Latin means, to all but the few, that even if accessible they are unreadable. Historians of science will be permanently in the author's debt for placing on record, in readable form, the results of his researches, which were carried out in many European libraries, as well as in those in the United States. It is perhaps inevitable that these volumes often read as if they represent the transcription of a series of tidied-up note-books, interspersed with the comments of the author. This is not to say, by any means, that the text is not easy reading, for it can be read with interest and pleasure; but it is regrettable that the author's conclusions on the numerous topics discussed are not summarized more often and more systematically.

"The History of Magic and Experimental Science" is primarily a source-book and a work of reference, and as such is likely to be consulted for many years to come. It is also a mine of bibliographical information. Use of the book is greatly facilitated by the elaborate index, contained in Vol. 8, which extends to 168 pages. It seems unlikely that the ground covered by Prof. Thorndike in these two volumes, and, indeed in the whole work, will ever again be re-traversed in such detail. As a history of magic it is outstanding; as a history of science it should be read in conjunction with the more formal histories of science. It is a pleasure to record that in December 1957 the author was presented with the George Sarton Medal, the highest award of the History of Science Society, for his numerous and valuable services to the history of science.

V. A. Eyles

[1] A second printing, with corrections, was published in 1929.
[2] *Isis*, **6**, 74 (1924).
[3] *Isis*, **23**, 471 (1935).
[4] Moore, L. T., "The Life and Works of the Honourable Robert Boyle", 214 (Oxford, 1944).

OLD CHEMISTRY

Chymia

Annual Studies in the History of Chemistry, Vol. 5. Edited by Henry M. Leicester. (Edgar F. Smith Memorial Collection: University of Pennsylvania. University of Pennsylvania and Division of History of Chemistry, American Chemical Society.) Pp. 214. (Philadelphia: University of Pennsylvania Press; London: Oxford University Press, 1959.) 40*s*. net.

It is six years since the previous volume of "Chymia" was published and it is good news that this attractive and valued selection of studies in the history of chemistry is again to appear annually. The American Chemical Society has furthered the interests of chemistry in a way which is internationally acknowledged and esteemed, and the inclusion of the history of chemistry in *Chemical Abstracts* for a long time has shown that it has this side of its many interests at heart. The editor of the present volume, well known for his valuable publications in the field, contributes an interesting paper on the spread of the theory of Lavoisier in Russia. The volume begins with a well-deserved tribute to the memory of Edgar Fahs Smith (1854–1928) and a list of his numerous publications relating to the history of chemistry, written by H. S. Klickstein. In a way, "Chymia" owes its existence to Edgar Fahs Smith, and his memorial collection in the University of Pennsylvania, left with an endowment, now includes part of C. A. Browne's collection and the library of Tenney L. Davis, making up more than nine thousand volumes and three thousand portrait prints.

The other papers cover a wide field, from ancient Mesopotamian metallurgy to the history of the structure of acetone. One of the longer papers, by Daumas and Duveen, describes Lavoisier's large-scale decomposition and synthesis of water in Feb-

ruary, 1785. There is an article by R. E. Schofield on Josiah Wedgwood as an industrial chemist and one by R. Siegfried on the chemical philosophy of Humphry Davy. American chemistry is represented by two papers, one on the Columbian Chemical Society by W. D. Miles and one by S. M. Edelstein on the chemical revolution in the United States as shown by the pages of the *Medical Repository*, a periodical initiated by Samuel Latham Mitchill in 1797. This paper is of interest in relation to Priestley, who published in Mitchill's journal.

One can always have too much of a good thing, when interest tends to decline. With such a relatively small publication as "Chymia", appearing once a year (as we hope it will), it follows that only material of the highest standard can find a place. It can be read, and since its field is restricted to chemistry it is not swamped by publications towards which chemists, with their generally practical and objective outlook, feel little drawn. Chemists can read and enjoy it, and even those who have little sympathy with historical studies as such cannot fail to find something in the pages of "Chymia" which will interest them. We all know who proposed the name "aldehyde". Who first used the name "acetone" ? A search of all the standard literature does not answer this question, but it is answered in this volume of "Chymia". Every chemist who up to now has not seen this fascinating publication is strongly advised to repair his loss.

J. R. PARTINGTON

PROPERTIES OF LIQUIDS

Liquids and Liquid Mixtures

By Dr. J. S. Rowlinson. (Modern Aspects Series of Chemistry.) Pp. ix+360. (London : Butterworths Scientific Publications ; New York : Academic Press, Inc., 1959.) 75*s.* ; 12.80 dollars.

DR. ROWLINSON'S book is admirable in many ways. In it is gathered together an impressive amount of quantitative information about the measured properties of liquids and liquid mixtures, and also a multitude of thermodynamics and statistical formulæ, including all those that are ever likely to be required for the correlation of purely thermodynamic properties. No reader could possibly complain that the author has paid inadequate attention to the experimental literature, or has failed to take seriously the difficulties which arise in the most advanced statistical theories.

The book, however, suffers from the defects of its qualities. It is a commonplace that nothing but trivialities can ever emerge from a purely thermodynamic discussion of homogeneous phases, and yet the statistical theory of liquids does not appear until two-thirds of the way through the book. One of the most valuable generalizations in the theory of liquids is the principle of corresponding states, and one would have thought that this principle, and its statistical basis, should have been introduced early on, as an invaluable first approximation from which real liquids, of course, deviate to varying degrees and for various reasons. Again, the thermodynamic properties of mixtures cannot be understood at all without reference to the intermolecular potentials, and surely the most satisfactory way of dealing with them is to interpret the data in terms of statistical models as one goes along. In this connexion it is a pity that nothing is said about the theory of Prigogine and his colleagues of 'ideal associated' solutions, or about the link-up between the spectroscopic and thermodynamic data on alcohol solutions. Further, though this is a fault of the title rather than of the book, there is virtually no mention of transport properties, or of other non-thermodynamic measurements which have thrown so much light on the liquid state.

The best chapters in the book are probably those on the critical state of pure liquids and liquid mixtures, and the final chapter on the statistical thermodynamics of mixtures of spherical molecules, which is based mainly on the work of W. B. Brown.

Finally, it is certain that the purely descriptive chapters on the phase diagrams of mixtures will be of educational value to the chemical engineer, for whom the moral of the book is "Anything can happen and it probably will".

H. C. LONGUET-HIGGINS

SOILS OF TANGANYIKA

A Survey of Soils in the Kongwa and Nachingwea Districts of Tanganyika

By B. Anderson. Pp. ix+120 (36 figures). (Reading : Department of Agricultural Chemistry, The University ; Dar-es-Salaam : Tanganyika Agricultural Corporation, 1957.) n.p.

EVERYONE knows the dismal sequel to the ambitious scheme for the mechanized production of ground-nuts in East Africa, which was characterized by the failure to employ pedological methods on pedological problems. After the horse had departed, some effort was made to shut the stable door and a very competent soil surveyor was set to work to make a proper study of the soils of the Kongwa and Nachingwea districts. The publication under review presents his results and shows what can be achieved by one trained pedologist working 'on the cheap' with limited facilities, but with specialized technical assistance from various institutions. The moral for would-be planners of land-use is obvious.

The first two chapters contain descriptions of the topography, geology, climate, vegetation and soils of the two areas. In each case the soils are described in detail and the soil patterns are shown on maps at 1/25,000. The latter are clear and pleasing to the eye, but the keys should have specified the parent material and topographic situation of each soil series, thus saving constant reference to the text.

At Kongwa the main catena consists of red earths developed on a sandy loam from granitic gneiss and brown and grey calcareous valley-soils on colluvium and alluvium. Some of the red earths are capable of cropping usefully in good years but the main limitation is the unreliability of the rainfall.

In the Nachingwea area, the major areas are occupied by a 'textural catena' developed by differential washing of a deep mantle weathered from more basic gneiss. The rainfall is nearly twice as high as at Kongwa and the wet season is somewhat longer.

The next chapter describes the soil maps in detail and clarifies the topographic relationships with cross-sections. In the last, there is a useful summary of ideas on the genesis of the soils and their relations to other tropical soils.

The book is well illustrated with photographs and diagrams and is a valuable addition to our knowledge of East African soils.

R. M. S. PERRIN

Acoustique Musicale

(Marseilles, 27, 28, 29 Mai, 1959.) (Colloques Internationaux du Centre National de la Recherche Scientifique, No. 84.) Pp. 259. (Paris: Editions du Centre National de la Recherche Scientifique, 1959.) 3,400 francs.

FROM time to time the subject of musical acoustics earns itself a colloquium. There was one recently in London (see *Nature*, **183**, 1293; 1959) and others have taken place in Switzerland and the United States. The colloquium the proceedings of which are under review was more ambitious, since from its audience it could be justly called international and was conducted at Marseilles, surprisingly under the patronage of the Centre National de Recherche Scientifique. Such gatherings usually attract a number of scientists who are amateur musicians but few professional musicians. The organizers of this one were fortunate in collecting an audience nearly equally divided between the two types. The papers presented indeed, with a few exceptions, fell into two classes, those concerned with the acoustics of musical instruments (the scientific) and those with intonation and musical scales (the arithmetic, if the writer of this notice may be pardoned for using the term). A surprising amount of 'academic' discussion was engendered concerning the effects of changing an interval in a scale by a small fraction say, a few cents, academic because real singers and instrumentalists do not hold intonation to such a precision.

One may have doubts whether this subject is an art or a science, but many readers of these proceedings will gain a refreshing insight into the range of interest and activity covered by the term 'musical acoustics'.

E. G. Richardson

Biological Sciences

Vol. 2. Edited by J. G. Bugher, J. Coursaget and J. F. Loutit. (Progress in Nuclear Energy, Series 6.) Pp. x+422+xii. (London and New York: Pergamon Press, 1959.) 105*s*. net.

THIS is an editorial selection of thirty-five papers out of the many presented to some of the biological and medical sessions of the Second Conference on the Peaceful Uses of Atomic Energy (1958). The subjects range from the distribution of labelled drugs in the body, to radiation-induced ageing in mice and induced mutations in polygenic systems. The reviewer can only apply a further arbitrary selection. A remarkable paper by Barnes, Ford, Gray and Loutit reports the discovery that repopulation of hæmopoietic tissue by host cells in radiation-chimæra is often effected by a very few clones of cells, sometimes recognizable by chromosomal 'markers'.

Ullberg gives some beautiful examples of how the distribution, localization and metabolism of certain (labelled) drugs can be followed by auto-radiography; Muller reviews the recent contributions of *Drosophila* work to the knowledge of radiation-induced mutation.

General papers deal with the 'ageing' effects of radiations and, more generally, with the effects on the life-span. Others deal with agents modifying the biological effects of radiations, with the elimination of radioactive substances from the body and with chemical effects of radiations on particular substances. A considerable proportion of the book is concerned with the induction of mutations.

The book is an indispensable—though somewhat expensive—reference for laboratories in any way concerned with the biological effects of radiations.

G. Pontecorvo

Tracer Applications for the Study of Organic Reactions

By John G. Burr, Jr. Pp. x+291. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1957.) 7.50 dollars.

IN this book the author has attempted to present the evidence obtained by the use of isotopic tracers in the study of organic reaction mechanism. A very wide field has been covered, and in some sections, for example, that on migration aptitudes, a valuable critical discussion has been included. It is rather a pity that this standard has not been adhered to throughout and the work thus becomes rather uneven. Its main value lies in the full literature index which forms a most useful guide to further reading on the many reactions discussed. The literature coverage (up to early 1953) makes certain sections out of date at the time of writing but the author feels that the book will be of some value to the advanced worker who wishes to use it as a literature source book. Valuable appendixes on the subjects of reaction mechanism and handling of isotopic tracers, together with an additional list of references covering the period 1953–1954, are also included.

A. I. Scott

Index of Chemistry Films

A Comprehensive List of Films and Filmstrips on Chemistry and Related Topics. Pp. ix+150. (London: Royal Institute of Chemistry, 1959.) 5*s*.

THE object of this publication is to provide an up-to-date guide to the eight hundred or so films and two hundred or so film strips on chemical topics that are available in Great Britain. The films are classified according to subjects. Brief information is supplied on how any film may be obtained, on its length, gauge, essential contents and, where known, suitability for particular audiences, especially schools. This is a most valuable pamphlet, the existence of which should be made widely known so that fuller use can be made by schools and other institutions of the extensive amount of available material.

Royal Institute of Chemistry Monographs for Teachers

No. 1: Principles of Electrolysis. By Prof. C. W. Davies. Pp. v+30. 3*s*. 6*d*. No. 2: Principles of Oxidation and Reduction. By Dr. A. G. Sharpe. Pp. v+30. 3*s*. 6*d*. (London: Royal Institute of Chemistry, 1959.)

EVERY student who goes up to university soon encounters the frustrating experience of having to 'unlearn' something of what he has been taught at school. There may well be something to be said for the educational value of varied approaches and the discarding, on the basis of more adequate arguments, of material acquired parrot-fashion at a younger age. There can be no doubt, however, that such lessons ought to be kept to a minimum and that time at school is in general more wisely spent on the teaching of fundamentals on a sound basis.

With this object in mind, the Royal Institute of Chemistry have now begun the issue of pamphlets on some of the more advanced topics of the school chemistry syllabus, to provide a short refresher course for teachers. The venture deserves every success and has had a good send-off with the two pamphlets issued so far.

V. Gold

GENETIC BASIS OF THE THALASSÆMIA DISEASES

By Prof. V. M. INGRAM and A. O. W. STRETTON
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IN recent years it has become clear that the abnormal human hæmoglobins, such as hæmoglobins *S*, *C*, *D*, *E*, etc., are variants of the normal protein (hæmoglobin *A*), differing from it by the substitution of perhaps not more than a single amino-acid residue by a new one[1]. These mutant forms are inherited as single genes. On the other hand, thalassæmia in both its minor and its major form, although inherited in a similar manner, has presented a much more complicated and confusing picture. In this anæmia, the inherited abnormality is not manifested in the production of recognizably abnormal forms of hæmoglobin, but rather in the apparent interference with the rate of production of the hæmoglobin *A* with consequent anæmia.

It is the purpose of this article to present and explore a unifying hypothesis—and an alternative—which seeks to explain the occurrence of thalassæmia and some of its interactions with the other hæmoglobinopathies in which chemically abnormal hæmoglobins are recognized. In particular, we wish to suggest that thalassæmia is a mutation producing a hæmoglobin with a 'hidden' amino-acid substitution, such that the hæmoglobin produced is electrophoretically normal. There are many such mutations possible. Some of the ideas which are contained in this theory have been talked about by many people and on numerous occasions (for example, Itano[2]); we do not wish to claim any great originality for them. It is hoped that the hypothesis, even if proved wrong, will serve to stimulate interest and direct experiment in this field.

Historical

Thalassæmia, or Cooley's anæmia, is a hereditary disease which occurs commonly in Mediterranean peoples, especially those of Italian and Greek origin. It is found in two forms, thalassæmia major, or Cooley's anæmia, and thalassæmia minor.

Clinically the major form of the disease is characterized by a marked hypochromic anæmia with abnormal red-cell morphology and the presence of target cells and oval red blood cells; the cells have increased mechanical fragility and osmotic resistance. The minor form, on the other hand, may be asymptomatic and in some cases is difficult to diagnose. Anæmia, if present at all, is only very slight; the hæmatological findings include increased target cells, microcytosis, and increased osmotic resistance of the cells.

Electrophoretic studies[3] and measurements of the rate of denaturation by alkali have shown that in the major form of the disease the proportion of a hæmoglobin having the mobility and behaviour of fœtal hæmoglobin is raised, sometimes as high as 97 per cent of total hæmoglobin. In the minor form the proportion of fœtal hæmoglobin is scarcely raised above the normal amount, which is of the order of 1 per cent. Rich[3] has suggested that fœtal hæmoglobin is produced to compensate for the marked decrease in the amount of normal adult hæmoglobin synthesized by people with thalassæmia major; if this is the case, it is apparent that the degree of compensation varies widely from individual to individual, as does the decrease in the rate of production of hæmoglobin *A*.

It is now well established that the parents of a person with thalassæmia major have thalassæmia minor, and extensive family studies have been carried out to support this[4]. However, cases have been reported where parents of thalassæmia major children do not show the usual findings associated with thalassæmia minor[5]. In addition to the increase in the proportion of fœtal hæmoglobin mentioned above, Kunkel[6,7] has recently shown a correlation between the proportion of a normal minor component of blood, hæmoglobin A_2, and the presence of a thalassæmia gene. Gerald and Diamond[8] have extended this in a survey of twenty-three parents of children with thalassæmia major. They found that in all these parents the proportion of A_2 is raised, the usual increment being from the normal 2·5 per cent to 5 per cent. However, Kunkel *et al.*[7] showed that two out of thirty-four parents of children with thalassæmia major had no increase in A_2, although examination of their blood suggested the presence of thalassæmia minor. This last finding suggests, as does the great variability of the disease, that there is not one kind of thalassæmia minor or major, but several and perhaps many.

Possible Molecular Mechanisms for Thalassæmia

The appearance of the cells in thalassæmia major suggests that they contain less hæmoglobin than normal. Perhaps such symptoms as are seen in the minor condition can be laid to this cause also. It is possible to explain the low hæmoglobin content in terms of a slower rate of hæmoglobin synthesis within the developing blood cell in a way similar to the explanation given by Itano[9] for the abnormal hæmoglobin diseases. If we assume that hæmoglobin synthesis is complete in all or almost all the cells in circulation, then the ratio of the amounts of different hæmoglobins in the same cell is equal to the ratio of their average rates of synthesis. In persons who are heterozygous for hæmoglobin *A* and for an abnormal hæmoglobin (for example, hæmoglobin *S*), the proportion of hæmoglobin *A* is usually higher than that of the abnormal, reflecting a lower rate of synthesis of the abnormal hæmoglobin. It has been suggested[9] that in cases of persons homozygous for an abnormal hæmoglobin or heterozygous for two abnormal hæmoglobins, the cells are microcytic and the blood contains numerous target cells. However, in sickle cell anæmia, the cells are normocytic, but this may be due to increased amounts of fœtal hæmoglobin in the cells. Since all the cells in sickle cell anæmia show the sickling phenomenon, even when hæmoglobin *F* is present, the synthesis of fœtal hæmoglobin and hæmoglobin *S* seems to occur within the same cells[10].

A slow rate of hæmoglobin synthesis in thalassæmia might be expected to result in hypochromic cells. We know of no direct experimental evidence

on whether fœtal hæmoglobin produced in thalassæmia major is in the same cells as adult hæmoglobin; but arguing from the findings in sickle cell anæmia, we would predict that they would be in the same cells. Experiments are needed to confirm this prediction and to compare the sites of formation of cells containing fœtal hæmoglobin in the normal fœtus and infant, in sickle cell anæmia and in thalassæmia major.

Possible Causes of a Low Rate of Hæmoglobin Synthesis

If the gene affected in thalassæmia is one which controls the production of an enzyme catalysing one of the many steps involved in hæm synthesis, a low rate of enzyme synthesis or a defective enzyme might result in this particular enzymatic reaction being the rate-limiting step in hæmoglobin production. The high serum iron and the saturation of the iron-binding capacity of the plasma have been taken as indications that inhibition in porphyrin synthesis is the basic defect in thalassæmia. However, on this basis it would be expected that the rate of synthesis of fœtal hæmoglobin should also be reduced in thalassæmia, if indeed it is true that fœtal and adult hæmoglobin are made in the same cells.

In the normal infant, a switch-over mechanism exists which causes fœtal hæmoglobin production to be replaced completely by adult hæmoglobin production within a few months of birth. The nature of this control mechanism is not understood; but if it were defective, fœtal hæmoglobin synthesis might not be arrested, as it is in the development of the normal infant, while at the same time the synthesis of hæmoglobin *A* might remain partially or completely unstimulated. Thalassæmia, in this view, would be a developmental defect, resulting in a state of immaturity of the hæmoglobin-synthesizing system.

The two mechanisms discussed above might be expected to result in a low rate of synthesis of adult hæmoglobin, and it is quite possible that some cases of thalassæmia are, in fact, caused by one or other of them. However, there is one aspect of the effect of a thalassæmia gene that is difficult to explain in these terms: it is found that in many cases where both clinical and family studies suggest the presence of both a thalassæmia gene and a gene for an abnormal hæmoglobin (for example, hæmoglobin *S*) in the same person, the proportion of abnormal hæmoglobin is roughly doubled in comparison to that found in a person singly heterozygous for an abnormal hæmoglobin. For example, in sickle cell or hæmoglobin *C* trait, the abnormal hæmoglobin constitutes roughly 40 per cent of the hæmoglobin, whereas in double heterozygosity for thalassæmia and hæmoglobin *S* or *C*, the proportion rises to about 80 per cent. The remainder is largely fœtal hæmoglobin. This indicates a specific inhibition of hæmoglobin *A* synthesis with little or no interference with production of the abnormal hæmoglobin. This differential effect is difficult to explain, if thalassæmia involves an effect on hæm synthesis, or a defect in the hæmoglobin *F* to hæmoglobin *A* switch mechanism, both of which should, *a priori*, inhibit also the abnormal hæmoglobin.

Studies on the ratio of hæmoglobin *A* to hæmoglobin *S* and to hæmoglobin *C* in heterozygotes have led Itano[11] to postulate that as well as containing the information for the amino-acid sequence of the globin, the hæmoglobin genes also have characteristic rate-determining information; thus, a gene controlling the production of a globin with a certain amino-acid sequence will effect synthesis of protein at a certain rate, while a gene associated with a globin having the same sequence except for one amino-acid may have a different rate of synthesis. For example, hæmoglobins *S*, *C* and *E* appear to be produced at a slower rate, and hæmoglobin *J*[9] at a faster rate, than normal.

Itano has also postulated[2], on the basis of the trimodal distribution of ratios of hæmoglobin *A* to hæmoglobin *S* in heterozygotes, that normal hæmoglobin is controlled by a series of at least three alleles, producing hæmoglobin at different rates; the hæmoglobins would have different amino-acid sequences, but the same electrophoretic mobility. We wish to extend these ideas to include thalassæmia, postulating that the 'hæmoglobin *A*' found in thalassæmia homozygotes has a different amino-acid sequence from 'normal', and that this new sequence determines a characteristically low rate of synthesis. If in some cases of thalassæmia major no hæmoglobin *A* appears to be produced at all, we would assume it to be due to a 'lethal' mutation similar to those found in micro-organisms where a mutation within a gene may cause no protein to be produced; so far only one such thalassæmia case has been described[3].

Amino-Acid Substitution Hypothesis

There are in the human at least three types of hæmoglobin: (*a*) the adult series of hæmoglobins (*A*, *S*, *C*, *D*, *E*, etc.); (*b*) the fœtal series (*F*, hæmoglobin 'Alexandra', etc.); (*c*) the normal minor components—for example, hæmoglobin A_2 of Kunkel and Wallenius[12].

Each form of hæmoglobin has, or probably has, four polypeptide chains of two different kinds[13,14], which we may write: Hb $A = \alpha_2{}^A\beta_2{}^A$; Hb $F = \alpha_2{}^{A'}\gamma_2{}^F$; Hb $A_2 = x_2{}^{A_2}y_2{}^{A_2}$ (to indicate that these have not yet been investigated). The subscripts have the usual chemical significance as in H_2O and the superscripts refer to the particular hæmoglobin. It has been shown that the α chains of hæmoglobin *F* are like the α chains of hæmoglobin *A* in gross structure[17,18]. When it is shown that they are alike in detail, then we will be able to write hæmoglobin $F = \alpha_2{}^A\gamma_2{}^F$. The possibility that these α chains are identical, and more important, the possibility that those chains are controlled by the same gene, are of great genetic, developmental and evolutionary significance. They also affect to some extent the hypothesis in this paper.

It is widely believed that the precise amino-acid sequence, and hence the structure, of each hæmoglobin peptide chain is controlled by a specific gene—with the proviso mentioned in the previous paragraph. For hæmoglobin *A* there would exist α genes to control α chains and β genes to control β chains. The genotype of a person making hæmoglobin *A* would be written as: α^A/α^A, β^A/β^A. The idea that two sets of genes might control production of hæmoglobin has been proposed before by Schwartz *et al.*[19] and by Smith and Torbert[20]. The genotype for fœtal hæmoglobin, and for A_2, can be written: $\alpha^{A'}/\alpha^{A'}$, γ^F/γ^F and X^{A_2}/X^{A_2}, Y^{A_2}/Y^{A_2}. The abnormal human hæmoglobins, in so far as they have been investigated, have proved to carry single amino-acid substitutions in one or other chain[1]. The molecule of the hæmoglobin *S* phenotype which has the mutation

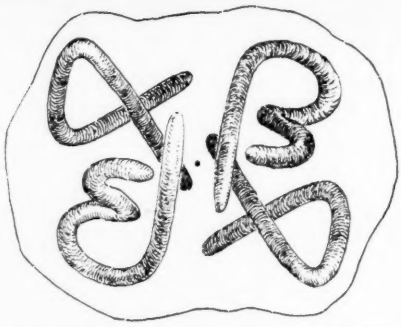


Fig. 1. Schematic diagram of the spatial arrangement of peptide chains in the hæmoglobin molecule. The sausage-like objects (ref. 16) represent the coiled polypeptide chains which are of two sorts: α peptide chains, beginning with the sequence valine–leucine, and β peptide chains, beginning with valine–histidine–leucine. They are arranged about a two-fold axis of symmetry (according to Perutz *et al.*; ref. 15) perpendicular to the plane of the paper

in the β chain[21,23] is written as $\alpha_2{}^A\beta_2{}^S$ and the genotype as α^A/α^A, β^A/β^S and α^A/α^A, β^S/β^S for the heterozygote and homozygote, respectively. Hæmoglobin *I*, which is an α chain mutation[21], would be written as $\alpha_2{}^I\beta_2{}^A$ and α^I/α^A, β^A/β^A in the heterozygote.

It is an important feature of the chemical work of establishing the phenotype of the abnormal hæmoglobins that both forms of the genotype find their phenotypic expression. The above sickle-cell heterozygote makes both hæmoglobin *A* and *S*. This is not necessarily so and one can well imagine instances where the mutation might be so drastic or of such a nature that the phenotype—the corresponding chain—cannot be made. We believe that some cases of thalassæmia may fall into this category.

We wish to propose that, in its typical form, thalassæmia is a mutation of the α or the β gene of the adult series, in no way different from those mutations which lead, for example, to hæmoglobin *S* and *C*, except that the consequent amino-acid alteration does not change the electrophoretic behaviour of the hæmoglobin or that the alteration is so drastic that this particular chain is not made at all and therefore never observed. So far we have confined ourselves to mutations which effect amino-acid substitutions at a point—point mutations. It will be obvious that the above second class of alterations, leading to non-production of the chain, could also be caused by other types of mutations such as deletions, inversions and substitutions involving a considerable number of amino-acids.

It is assumed for the time being that the four genes involved in, say, α^A/α^A, β^A/β^A, act independently and produce the chain peculiar to them. Thus, the genotype α^I/α^A, β^A/β^S, if it existed, would produce: $\alpha_2{}^I\beta_2{}^A$ = Hb *I*; $\alpha_2{}^A\beta_2{}^A$ = Hb *A*; $\alpha_2{}^A\beta_2{}^S$ = Hb *S*; $\alpha_2{}^I\beta_2{}^S$ = Hb *X* as yet unknown. The elegant re-association experiments by Singer and Itano[22] and of Vinograd, Hutchinson and Schroeder[23] have shown that real hybrid molecules, such as $\alpha^I\alpha^A\beta_2{}^A$, for example, do not occur naturally and cannot so far be made *in vitro*. It is also possible that a metabolic link exists between the α and the β gene derived from a particular parent, such that the genotype α^I/α^A, β^A/β^S makes only hæmoglobin *I* and hæmoglobin *S*. There is as yet no definite evidence against this idea, but it seems to us unlikely.

One important consequence of the theory is that thalassæmias, which have always been diverse in characteristics and distribution, can now be divided into two classes affecting the α and the β gene. However, within each class, great diversity is just what one would expect, because the number of possible mutations leading to 'non-electrophoretic substitutions' must surely be very large. They can differ in the particular amino-acid affected provided that such changes do not lead to alteration in electrophoretic behaviour, and consequently they can differ in the extent to which the rate of synthesis has been altered. On this basis the 'thalassæmias' range from mutant forms which are almost indistinguishable from the normal, even by chemical examination, to those in which the mutation is so drastic—lethal—that the hæmoglobin is not made at all. The genotypes of such thalassæmias might be written as α^A/α^X, β^A/β^X or α^A/α^A, β^X/β^A, with many different alleles. (Superscript '*X*' refers to the altered gene.)

Although many such thalassæmias are theoretically possible, in practice they need not form a continuum. Only some may be clinically important and therefore recognizable.

One of the appealing consequences of the hypothesis is that it provides an acceptable explanation of the cases where the thalassæmia gene, when present together with a single hæmoglobin *S* gene, suppresses the formation of hæmoglobin *A* but not that of hæmoglobin *S*. Previous ideas which postulated that thalassæmia is a suppression of hæmoglobin synthesis have come up against the difficulty that this suppression can act selectively against hæmoglobin *A*, but not against the abnormal hæmoglobins such as *S*, *C* and *E*; this is in spite of the fact that each of these differed by only 1 out of 280 amino-acids[24] from hæmoglobin *A* and from each other. We have always found such a situation difficult to visualize. If, on the other hand, thalassæmia is a mutation of, say, the β gene, then a sickle cell-thalassæmia might have a genotype such as α^A/α^A, β^X/β^S. This phenotype would not have the genetic equipment to make any hæmoglobin *A*, but would produce hæmoglobin *S* together with a reduced amount of $\alpha_2{}^A\beta_2{}^X$ (electrophoretically identical to $\alpha_2{}^A\beta_2{}^A$). As a compensating mechanism he would make hæmoglobin *F* ($\alpha_2{}^A\gamma_2{}^F$), which has γ chains instead of β chains. Alternatively, the genotype might be α^X/α^A, β^A/β^S if the subject has an α chain thalassæmia. In this case, synthesis of apparently normal hæmoglobin *A*, which is really $\alpha_2{}^X\beta_2{}^A$, might go on to a small extent in addition to production of hæmoglobin *A*, depending on the severity of the mutation. Also the degree to which increased production of hæmoglobin *F* is allowed or required might be expected to be very different in this second case, assuming a common α gene for hæmoglobins *A* and *F*.

If the same α gene controls the production of the α chains of hæmoglobin *F* and the α chains of hæmoglobin *A*, then individuals homozygous for severe α thalassæmias should not be found. *In utero* they would have very low levels of hæmoglobin *F*, which would most likely prove fatal in the absence of any compensatory effect. This conclusion implies that all patients with thalassæmia major are either homozygous for a β-chain defect, which would not interfere with the production of fœtal hæmoglobin, or that they are doubly heterozygous for α- and β-chain defects, or else that they have a defect of a different type.

If it should be the case that Kunkel's hæmoglobin A_2 also has α chains, which are similar to those of hæmoglobins *A* and *F*, and if all these α chains are

controlled by one and the same 'α gene', then the two types of thalassæmia would be expected to differ in the extent to which the A_2-level should be affected. A β chain mutation would allow an increase in A_2 to occur, whereas an α chain mutation would not. Such differences in the level of A_2 in different thalassæmias are known to exist. Nevertheless, it would probably be unwise to throw out entirely the notion that thalassæmia can act as a general suppressor of hæmoglobin synthesis. The disease exists in so many varied forms that some of them may be of this type. We are postulating that the majority of thalassæmias fall into the class described by the 'substitution' hypothesis.

Application of the Hypothesis

Silvestroni and Bianco[25] describe two families including a member having 'microdrepanocytic disease'—or sickle cell-thalassæmia disease (see Fig. 2).

The genotypes were deduced from hæmatological examinations; details are not given in the paper, so the phenotypic expression of the thalassæmia gene in the thalassæmia minor cases is not known; the proportions of different types of hæmoglobin were not measured. We notice two marriages involving a person heterozygous for both thalassæmia and sickle cell trait. In one case ('family 6', Fig. 2), such a man when married to a normal woman has a doubly heterozygous child, and in the other case ('family 9', Fig. 2) when married to a woman with thalassæmia minor has a normal child. The *S* change is known to occur on the β chain of hæmoglobin[21,23] and we assume this to be a case of the same hæmoglobin *S*. These patterns of inheritance and the other statistics presented by the authors suggest that the gene for thalassæmia and the gene for sickle cell anæmia in these double heterozygotes are non-allelic and the pattern of inheritance can be explained as follows by assuming the thalassæmia to be of the α variety (Fig. 3). Since we cannot deduce whether the mother of 'family 9', who has thalassæmia minor, has an α or a β thalassæmia, the child with thalassæmia major in this family could either have two α gene defects, or an α and a β defect. If the genes produce the appropriate chains independently, we would expect the double heterozygotes $\alpha^X/\alpha A$, $\beta A/\beta S$ to produce hæmoglobin *A* in addition to *S*. The reported data are not sufficient to corroborate this deduction.

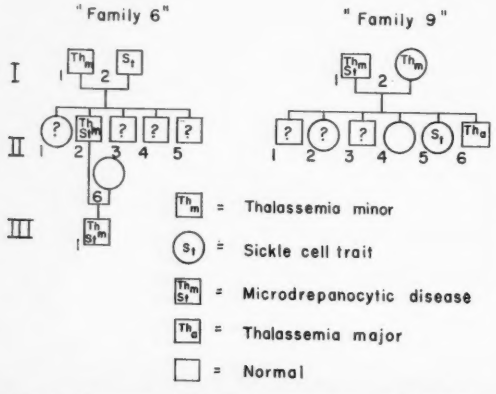


Fig. 2. Phenotypes of the living members of two of the families studied by Silvestroni and Bianco (ref. 25)

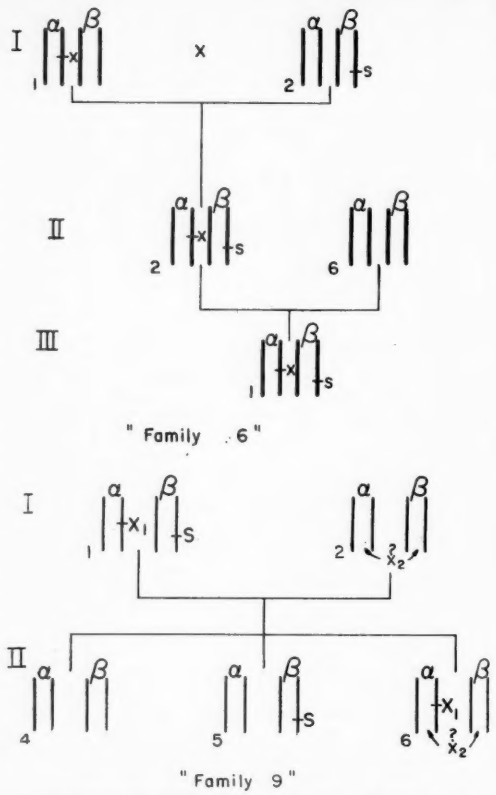


Fig. 3. Genotypes of the families shown in Fig. 2, but including only individuals with known phenotypes. The vertical lines represent the α and β hæmoglobin genes as marked: + *S* denotes the sickle cell mutation, and + *X* the thalassæmia mutations

Neel[26] described a family where a normal person and a person who appears to be heterozygous for both sickle cell anæmia and thalassæmia have two apparently normal children, one child and possibly a second with thalassæmia minor, one and possibly a second with sickle cell-thalassæmia disease, and one child with sickle cell trait. In order to explain this in terms of the two hæmoglobin genes, the thalassæmia defects must in this case be on the α gene; this family, 'kindred 60' (Fig. 4), provides evidence for the idea that this thalassæmia is on the α gene, while the hæmoglobin *S* mutation is on the β gene. The last series for sickle cell-thalassæmia disease are three families described by Ceppellini[27], where the children of a normal person married to a person with sickle cell-thalassæmia disease are either thalassæmic or have sickle cell trait. In one family of seven, three children have sickle cell trait and four have thalassæmia minor, while the other two families include three children each, and in both cases two have sickle cell trait and one has thalassæmia minor. In these three families involving thirteen children, six have thalassæmia minor and seven have sickle cell trait, which suggests that the thalassæmia defect is in these cases on the hæmoglobin β gene, like hæmoglobin *S*, since all the children inherit either sickle cell trait or thalassæmia minor, with equal probability; it is, of course, impossible to reach definite conclusions using such small numbers. The critical

family of Schwartz *et al.*[19], which includes cases of hæmoglobin *S* and *G* as well as thalassæmia, is under re-evaluation and will not be discussed here.

Hæmoglobin H. It was recently reported[28] that hæmoglobin *H* is composed of four polypeptide chains, but that these are all of the β variety. This agrees well with the physical behaviour and the instability of this hæmoglobin.

According to Motulsky[29], such cases occur together with thalassæmia minor in the family, and therefore it was postulated that "hæmoglobin *H* is only shown electrophoretically in the presence of thalassæmia". On the other hand, Bingle *et al.*[30] have reported hæmoglobin *H* without apparent thalassæmia.

Taking the first cases, two explanations are possible. If it is true that the β chains of which hæmoglobin *H* is composed are in every detail normal, then we may postulate that hæmoglobin *H* occurs where two mild α thalassæmias occur together (Fig. 5*b*). One of these might be so mild as to be not noticeable until together with the other. The genotype $\alpha^{X_1}/\alpha^{X_2}\ \beta^A/\beta^A$ would manufacture a large excess of β chains which complex to form β_4^A = hæmoglobin *H*. In Fig. 5*a* the person III 1 shows hæmoglobin *H* and II 3 and III 4 show thalassæmia. In the alternative explanation, the hæmoglobin *H* β chains might have a non-electrophoretic change such that production of this chain is increased or such that when the proportion is increased due to an α thalassæmia, the β^H chains polymerize preferentially. In this case the genetic picture would be as shown in Fig. 5*c*.

In two families described by Fessas[32], hæmoglobin *H* is transmitted from father to son, showing that the genes for hæmoglobin *H* and thalassæmia cannot be allelic, since they are both inherited from one parent. In these cases, the second explanation, namely, that a non-electrophoretic change in the

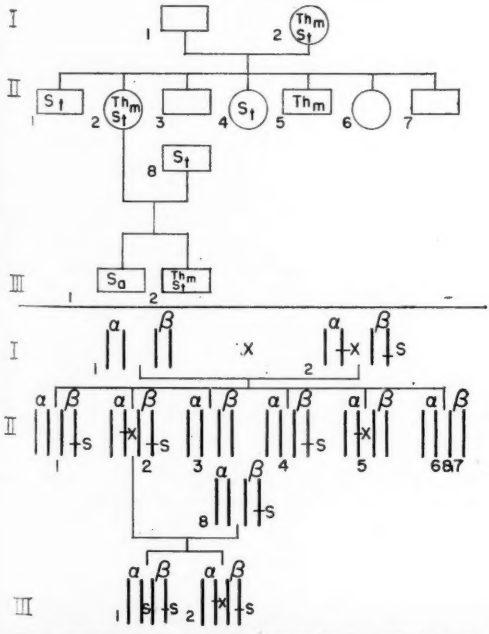


Fig. 4. Genotypes and phenotypes of individuals in 'Kindred 60' of Neel (ref. 26)

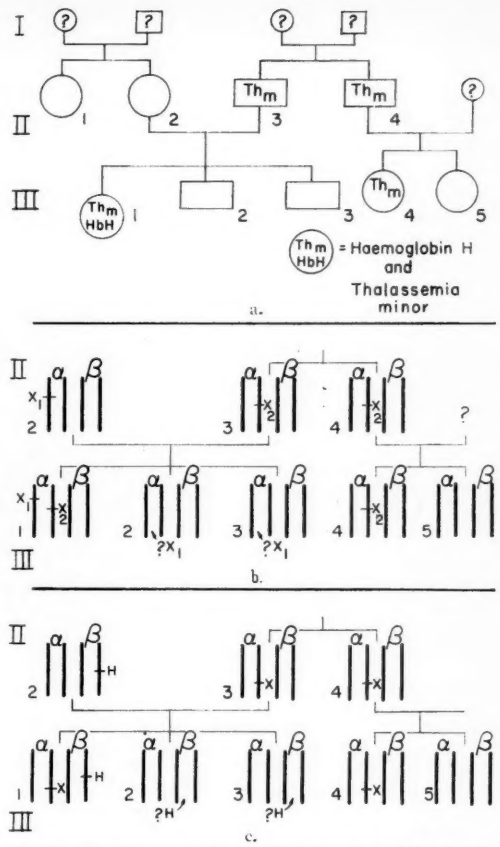


Fig. 5. Family described by Gerald (ref. 31) illustrating the inheritance of hæmoglobin *H*. *a* = phenotypes; *b* and *c* = the two patterns of inheritance discussed in the text

β chain allows a preferential polymerization of excess β chains, must be preferred.

It might equally be expected that a hæmoglobin composed of four α chains could be produced in circumstances similar to those leading to formation of hæmoglobin *H*, namely, the presence of two mild thalassæmia genes affecting the rate of synthesis of β chains, thus:

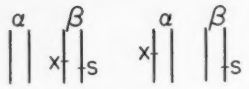
$$\alpha^A/\alpha^A,\ \beta^{X_1}/\beta^{X_2} \rightarrow \alpha_2^A\beta_2^{X_1} + \alpha_2^A\beta_2^{X_2} + \alpha_4^A$$

Alternatively, an altered α gene might produce an α chain which could polymerize to give α_4^A, if α chains tend to be produced in excess due to thalassæmia.

Discussion

Consideration of the families of patients with sickle cell-thalassæmia disease in terms of the hypothesis that the hæmoglobin gene is really two genes has led us to conclude that the thalassæmia defect, producing a hæmoglobin having the same electrophoretic mobility as normal hæmoglobin, but at a reduced rate, may in some cases be located on the hæmoglobin β gene (Fig. 6, case 1), and in other cases is on the hæmoglobin α gene (Fig. 6, case 2).

In case 1, since both copies of the hæmoglobin β gene are altered, one copy producing an electrophoretically distinct polypeptide chain, and the other



Case 1 Case 2

Fig. 6. The two possible genotypes to be expected in sickle cell-thalassæmia disease

producing a pseudo-normal chain but at a greatly reduced rate, it might be expected that the proportion of hæmoglobin *S* to 'normal' hæmoglobin should be higher than in the case of sickle cell trait ; this is observed in most cases.

In case 2, however, one copy of the hæmoglobin α gene and one copy of the hæmoglobin β gene are quite unchanged, so the proportion of hæmoglobin *A* would be expected to be higher than that of hæmoglobin *S*, if $(\beta^A)_2$ is produced at a higher rate than $(\beta^S)_2$ as we have supposed. It is significant that in the case of Neel's family (kindred 60) where the genotype can be deduced to be type 2, the proportion of hæmoglobin *S*, as determined by later analyses, is 22, 28 and 36 per cent in three individuals, in contrast to 60–80 per cent found in other cases of sickle cell-thalassæmia disease[34] ; further data on this point are needed. Furthermore, re-examination of this family[33] (kindred 60) has shown that the A_2-level was not elevated in individuals where the presence of a thalassæmia gene has been deduced. If A_2 has an α chain and the α gene responsible for its production is identical to that of hæmoglobin *A*, such an α thalassæmia would not be expected to allow the proportion of A_2 to rise, whereas a β thalassæmia, while decreasing the rate of synthesis of hæmoglobin *A* by lowering the rate of synthesis of β chains, should allow the level of A_2 to rise, if the α chains only are common.

In this connexion it is interesting to note that in two cases of thalassæmia-hæmoglobin *C* disease, where the genetic data are insufficient to establish the probable genotype, in one family hæmoglobin *C* is present to the extent of 74–77 per cent[35], while in the other family it comprises only 29 per cent[36] of the total hæmoglobin. This difference in interaction of the two genes may be due to the presence of different thalassæmia genes in each case, one on the hæmoglobin β gene and the other on the hæmoglobin α gene, respectively, as postulated for different cases of sickle cell-thalassæmia disease.

Further examination[37] of the second family in the previous paragraph, where the thalassæmia is of the 'non-interacting' kind, showed that the parent from whom the thalassæmia gene was transmitted did not have an elevation of A_2. This was also true of other children of the same parent, who also showed all the other characteristics of thalassæmia minor. On the other hand, persons with thalassæmia minor in the first family, with the 'interacting' type of thalassæmia, showed in the original electrophoretic patterns an 'unidentified component', later identified as hæmoglobin A_2 (ref. 38) at levels of 6–7 per cent.

It would be interesting to investigate the proportion of hæmoglobin A_2 in individuals with thalassæmia minor in these families where different sorts of genotypes are proposed. In many cases of thalassæmia minor, the level of A_2 is roughly doubled, as is usually the case with the level of abnormal hæmoglobin in double heterozygotes ; it is possible that α and β thalassæmias may differ by allowing or not allowing the proportion of A_2 to rise in compensation. In this connexion the findings of Kunkel *et al.*[7] where parents of children with thalassæmia major were found having A_2-levels within the normal range are very interesting.

It is also important to remember that cases of parents of children with thalassæmia major have been found who carry none of the characteristics of thalassæmia minor, so apparently normal people may, in fact, carry a thalassæmia gene[5,39].

This hypothesis as to the possible genetic and molecular mechanism involved in thalassæmia is open to experimental test ; it would predict that in cases of thalassæmia major the hæmoglobin having the same mobility as hæmoglobin *A* has a different amino-acid sequence, possibly bearing the same relationship to normal hæmoglobin as do the established 'abnormal' hæmoglobins that have been studied in detail ; namely, a difference in one amino-acid. Such a difference might be detected by analysis of the amino-acid composition of peptides of hæmoglobin *A* from normal and thalassæmia major subjects.

It may be that the wide variability in expression of thalassæmia is due to the possibility of the simultaneous presence of different types of genetic defect. For example, the genotypes α^{X_1}/α^A, β^A/β^{X_2} and $\alpha^{X_1}/\alpha^{X_2}$, β^A/β^A might lead to different phenotypic expressions and to different severity of the disease. Bearing in mind the further possibility of a defect in hæm synthesis, or other abnormalities, a wide spectrum of clinical states might be expected.

An Alternative : the 'Tap' Hypothesis

Freese[40] put forward the concept of chromosome structure in which genetically significant deoxyribonucleic acid alternates in a linear arrangement with 'connecting units'. Based on this idea, we can construct a second hypothesis to explain the occurrence of thalassæmias. Freese's 'connecting units' could be made of protein or of deoxyribonucleic acid and the structure of these units, if protein, could be determined by a small part of an adjacent deoxyribonucleic acid template. It might be further assumed that these connecting units serve to activate or inactivate the genetic deoxyribonucleic acid which follows them. In the case of the β chain gene, it might happen that a defect in the connecting unit preceding it results in this gene remaining silent. Therefore, β chains are not made and a β chain thalassæmia results with its deficiency in hæmoglobin. Such a defect would be genetically controlled by an adjacent region of deoxyribonucleic acid, sufficiently close to the β chain gene to be indistinguishable by the presently available data in human genetics. A similar picture would hold in the case of α chain thalassæmias. The defect in activating the α or β chain genes might be expected to range from zero to complete shut off, producing a number of intermediate steps. This explanation in which the 'tap' is turned off differs fundamentally from the one presented in the previous sections, because here the α or β chain genes are assumed to be perfectly normal. It is merely the degree to which they are activated that is affected. When, under the 'tap' hypothesis, a chain is made at all, its amino-acid sequence would be normal, even if it is made more slowly. On the previous hypothesis, the 'substitution' hypothesis, the chain when made is expected to carry one or more amino-acid substitutions or deletions. The genetic analysis presented in the preceding

section is valid in terms of both hypotheses since the gene and its 'tap' would be very closely linked. We have chosen to develop the 'substitution' hypothesis in greater detail because it is based on fewer assumptions than the 'tap' hypothesis.

Summary

We propose that thalassæmia is a mutation of either the α or the β hæmoglobin gene, of the same kind as that observed in the abnormal adult hæmoglobins, but without effect on the electrophoretic behaviour of the protein. Such mutations would be expected to reduce, sometimes to nothing, the rate of production of that hæmoglobin.

We are grateful to Dr. Park Gerald, Boston, and Dr. H. Lehmann, London, for the help of many stimulating discussions. We wish to express our thanks to the Medical Foundation, Inc., Boston, for a grant, and to the National Institutes of Health for a fellowship (A. O. W. S.).

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INTERNATIONAL MINERALOGICAL ASSOCIATION

FIRST GENERAL MEETING

MINERALOGISTS from nineteen countries assembled in Zurich at the end of August to attend the first meeting of the International Mineralogical Association and afterwards to go on one of the two field excursions. Many of those present had also attended the foundation meeting of the Association at Madrid in April 1958. There it had been laid down that the Association shall be an association of national mineralogical societies and pursue the aim of furthering the cause of mineralogy in all its aspects. To effect this, technical problems confronting mineralogists will be dealt with by special commissions, while scientific matters will be treated in symposia and eventually in publications sponsored by the Association. The commissions (at present numbering four) proceeded to deal with questions relating to abstracts, new minerals, mineral data and museums, while at business meetings the statutes of the Association came up for discussion and amendment. Two symposia were held of which a brief account will be given here. The papers will be printed *in extenso* in a coming volume of the *Estudios Geologicos* published by the Instituto "Lucas Mallada" in Madrid.

Alpine Mineral Fissures

The first symposium, held under the chairmanship of Prof. E. Wenk (Basle), dealt with Alpine mineral fissures. To these may be assigned numerous occurrences in Switzerland and also some in quite different regions. Prof. R. L. Parker (Zurich) outlined the main features of these deposits as shown by the Swiss localities. Though deservedly called "Alpine", the distribution in the Alps of such occurrences is localized and restricted to areas in which the interplay of mechanical forces and strength properties of the rocks is suitably balanced. The resulting fissures are late- to post-tectonic in formation and filling and show no prolongation in depth. They contain varied mineral assemblages which are considered to have crystallized from solutions deriving their contents from the surrounding rocks. The belief that this process (known as lateral secretion) was widespread in the Alps is supported by the quite general dependence of the fissure contents on the character of the country rock and frequently also by the presence around fissures of a zone of leached rock which seems to have been the source of the matter now contained in the crystallized minerals of the fissure. Among these, quartz and adularia are practically ubiquitous.

The relationship of Alpine fissure minerals to those of the druses in the granite of Baveno was discussed by Prof. G. Fagnani (Milan). That such a comparison may be made is shown by the numerous minerals common to both types of occurrence. These include not only widespread species such as quartz and

orthoclase but scarcer ones also, and even some like gadolinite and bazzite that are rarities in central Europe. In spite of such similarities, the Alpine fissures and the Baveno geodes actually have little in common. Even the minerals occurring in both show differences in habit which reflect the differing modes of formation. This is especially true of orthoclase, which in the Alpine fissures is always developed as adularia which never appears at Baveno. The Baveno quartz, though well crystallized, lacks the wealth of forms prevalent in the fissures and never shows the Ticino habit with large acute rhombohedra found in some Swiss occurrences. A significant paragenetic detail is the appearance of zinnwaldite instead of muscovite as chief mica of the geodes.

Dealing with the origin of quartz as a fissure mineral of the Alpine type in the U.S.S.R., Prof. D. P. Grigoriev (Leningrad) stated that numerous mineral occurrences in the Soviet Union are assigned to the Alpine type of fissure deposit. A reason for doing so is the possibility of establishing definite connexions between the country rock and the mineral parageneses found in the veins and cavities in question. Analytical results obtained on unaltered country rock and on altered specimens from the veins provide corroborative evidence. As a special instance of Alpine-type mineral genesis, Prof. Grigoriev discussed the derivation and crystallization of quartz found in veins which are younger than the quartzite rocks in which they occur. Many of the observed peculiarities of Alpine quartz relating to crystal habit, distortion, aggregate development, distribution, etc., have been traced to and explained by such factors as the number, distribution, and exposure of seed crystals, and the physical state of the circulating solutions, which may be saturated in respect to the large seed crystals of quartz while still unsaturated as regards the small ones. The relative rates of crystallization of different minerals were also discussed and prove to be characteristic for a given locality though varying strongly from place to place.

Prof. M. A. Kashkai (Baku) showed that in the Caucasus Mountains (which possess many points of similarity to the Alps) mineral deposits frequently occur which, judged by the applicable criteria, must be considered to be of a nature and formed by processes akin to those of the Alpine fissures. Emphasis was laid on the wide distribution of so-called 'listvenites', which are metamorphic rocks consisting chiefly of quartz, carbonates, and chlorite besides numerous accessory constituents. As these are sometimes formed from hydrothermal solutions, relationships between them and deposits of Alpine type can be established.

Prof. J. Lietz (Hamburg) described experiments he has carried out on small cubes of colourless quartz designed to convert them into smoky quartz by electrolysis. The cubes are placed between electrodes and treated in an electric field of 200–1,500 V./cm. at temperatures of 700°–900° C. The arrangement of the electrodes fundamentally affects the results obtained. When the crystallographic axis of the crystal lies in the direction of the field and each electrode completely covers one side of the cube, a front of colour appears at the anode and migrates towards the cathode, thus progressively filling the entire cube. However, when the front has reached the cathode, the cube starts losing colour at the anode and finally becomes colourless again. When the anode only partially covers one side of the cube, the portion of the latter lying immediately in front of the anode is coloured and discoloured as just described; whereas that part of the cube not covered by the anode also assumes an intense dark colour which does not disappear and proves to be thermally stable up to 1,000° C. The phenomena agree well with the assumption that colour centres exist in the quartz lattice in the form of $(AlO_{4/2})$ tetrahedra which yield an electron when electrolysed.

Prof. F. Laves (Zurich) spoke on the feldspars of the Alpine fissures and their twinning. While the twins of adularia (potassium-feldspar with up to 10 per cent sodium) consist of a restricted number of individuals, albite (translucent sodium-feldspar) tends towards polysynthetic twins comprising many individuals. Such twinning may be the result of diffusive changes in the distribution of aluminium and silicon in the lattice or of displacive transformations due to a temperature-conditioned collapse of the structure. However, polysynthetic twins sometimes result from mechanical deformation when the distribution of aluminium and silicon is sufficiently disordered. The more opaque milky white variety of albite elongated after the *b*-axis and known as pericline is twinned according to a law usually defined as a rotation of 180° about the *b*-axis. Investigations show, however, that structural changes must have taken place after twin formation and be the reason why the inter-relation between the crystals only approximates to this law, the 'twin axis' being actually an irrational direction in (001). Leaching of a primary anorthite content and its partial metasomatic replacement by albite substance is a reason for this condition and causes numerous minute voids within the crystals. This accounts for their milky appearance.

Twinning of Crystals

The second symposium, the chairman of which was Prof. Martin J. Buerger (Cambridge, Mass.), had as its topic the twinning of crystals. Prof. Buerger's own paper was devoted to various aspects of twinning in general but with special regard to the boundaries between the twinned individuals. While the low energy of such junctions is quite generally a most characteristic feature of twins, the latter may be connected either by coherent or incoherent boundaries. A common type of coherent twin results from simple or more general stacking faults in the structure. Ex-solution pairs, however, always have incoherent boundaries. According to Prof. Buerger, twins related by 3-fold, 4-fold and 6-fold rotations are certainly possible.

Dr. H. Curien (Paris), discussing the degree of symmetry which a twin axis may possess, recalled the belief held by Georges Friedel that twin axes may be of a 2-, 3-, 4-, or 6-fold character. Examining each of these possibilities, Dr. Curien considers unlikely those involving 3- or 6-fold rotations; for these could not bring about any strict coincidence of lattice planes belonging to the two participating crystals. An example believed by Friedel to exemplify twinning by such an operation is the sagenite twin of rutile in which three crystals seemingly occupy positions realizable by a 60° rotation around the *a*-axis. However, this grouping can be accounted for by supposing the well-known twin laws of rutile (reflexion on (011) and (031)) to be both operative. They lead to positions deviating by only 10′, that is, by an amount within the experimental error, from the theoretical hexagonal positions. Dr. Curien accepts the possibility of twinning according to 4-fold axes.

These will be oriented along lines of intersection between perpendicular planes present in all systems except the triclinic. Such axes will, however, not produce actual coincidence of the nodes of the lattice planes, so that the 'quality' of such twins must be much inferior to that of ordinary ones.

Prof. J. D. H. Donnay (Baltimore) proceeded from the fact established by Georges Friedel that a crystal twins whenever there is a superlattice that has (rigorously or approximately) more symmetry than the actual lattice of the crystal. Similarly, it seems that the presence of an appropriate sub-lattice may lead to twinning. It was then shown that twinning may also be explained in terms of the 'lattice complex' of Paul Niggli. This term is applied to the assemblage of all like atoms in a structure independently of its position in the space group. When such a complex of atoms has more symmetry than the space group as a whole, the remaining atoms can distribute themselves in various ways which are related by symmetry operations belonging to the complex but not to the space group. These are the operations by which certain types of twinning are achieved.

Dr. P. Hartman (Groningen) spoke on the twin of atacamite, the law of which is definable either as a 120° rotation about the [950] direction or as one of 180° about [544]. The structural explanation lies, according to Dr. Hartman, in the parallelism of chain links in the structure and not of lattice rows as in ordinary twins. This, however, recalls the manner in which crystals of different species sometimes grow together in oriented pairs. This phenomenon is known as epitaxy, and Dr. Hartman suggested that very close relationships exist between it and twinning. This would imply the non-existence of 3-, 4-, and 6-fold twin axes.

Dr. W. T. Holser (La Habra, Cal.) analysed the relations of symmetry and structure at the boundary plane of a twin, extending his investigation to include trans-boundary relations. He re-described Buerger's model of a trans-boundary growth step as a particular type of screw dislocation. Twin stability is related to some measure of fit along the dislocation of the step, which latter may be symmetrical or pseudo-symmetrical.

Prof. T. Ito (Tokyo) spoke on twinning as a building principle of crystals and introduced the new concept of 'space groupoids' as distinct from space groups.

Dealing with twinning in diamond, Prof. A. Neuhaus (Bonn) first summarized some of the conflicting observations made on this mineral. As a result of his own investigations, he considers it certain that a transition between two modifications takes place but believes that only one is isometric. He assigns this one (α-diamond) to the O_h crystal class while attributing rhombohedral (D_{3d}–) symmetry of pseudo-isometric character to the other (β-diamond). Coarsely lamellated diamond seems to have evolved by natural tempering from such material showing finer alternations and arising from the transformation of the original isotropic substance. Thus a distinction must be made between the true double-refraction of β-diamond and irregular effects due to strains produced by (sub-) microscopic inclusions. Primary α-diamond is stable at high pressures and temperatures above 1,500° K., whereas β-diamond is stable at lower temperatures. Natural diamonds always belong to the β-modification and may either have been formed as such from the outset or be the result of a secondary α-β transformation. In either case twin lamellæ will be present which are primary in one and secondary (mimetic) in the other. Prof. Neuhaus demonstrated a new phase diagram of carbon from which conclusions regarding the stable formation of diamond can be drawn and the differences between Type-1 and II diamonds explained.

Dr. I. Sunagawa (Tokyo) described the growth and natural etch patterns observed with a phase-contrast microscope on the boundaries of hematite crystals forming contact twins after (10$\bar{1}$0). The surface structures of the twin boundaries are difficult to explain as simple junctions in twin position effected during growth. It seems, rather, that the mechanism of contact twinning involves processes of faulting or slipping of the participating lattices.

ROBERT L. PARKER

SCHOOL OF PHYSICS WITHIN THE UNIVERSITY OF SYDNEY

THE research activities and student numbers of the School of Physics within the University of Sydney, the head of which is Prof. H. Messel, have expanded so much during the past six years that a major reorganization has become necessary.

Henceforth there will be five departments within the School; the newly created departments are: (1) The Basser Computing Department; (2) The Daily Telegraph Theoretical Department; (3) The Falkiner Nuclear Department; (4) The Wills Thermonuclear Department; (5) The Chatterton Astronomy Department.

The Basser Computing Department arises from the Adolph Basser Computing Laboratory with the electronic digital computer, *Silliac* I. This computer has been a great success in Australia and largely responsible for opening up the computing field there. Opened on July 1, 1956, *Silliac* I has run at high reliability since that date, and at present is occupied 24 hr. a day on a three-shift basis. Work has or is being carried out for every University in Australia, two in New Zealand, for 20 government and semi-government organizations, and 87 private organizations. Some two hundred personnel throughout the country have attended courses on the computer and a postgraduate diploma course in automatic computing has been established.

Steps are now being taken to proceed with the construction of a much faster transistorized machine. *Silliac* I was constructed using the University of Illinois design of *Illiac*. The new computer in the Basser Department is likely to use the design of the new fast computer at present being built by the Illinois group. The present cost is estimated at £A.575,000. These funds have still to be provided.

Dr. John M. Bennett is in charge of numerical analysis and the user end of *Silliac* I, while Mr. B. E. Swire is chief engineer.

The Daily Telegraph Theoretical Department was established with a gift of £A.50,000 from Consolidated Press, Ltd. The theoretical group which forms the basis of the newly created department always has been a strong one. The appointment of Prof. S. T. Butler to the chair of theoretical physics should do

much to enhance the position of the Department. Work in the Department is centred on nuclear, solid-state, low-temperature, shower and field theory.

An original gift of £A.50,000 by Dr. G. B. S. Falkiner was used to establish the F. B. S. Falkiner Nuclear Research Laboratory in memory of his father. This Laboratory, with twenty scientists, has been working during the past six years on various aspects of cosmic radiation and forms the basis of the new department. At present there are two large groups. One is specializing in the use of nuclear emulsions for the study of the primary cosmic radiation and high-energy jets; the second group is concerned with a large-scale study of extensive air showers.

Considerable expansion of the efforts within the Department have taken place since the appointment of Prof. C. B. A. McCusker as leader of the cosmic-ray team of workers. Further expansion in the work of both the emulsion and extensive air shower groups is taking place at present.

The equipment from the large Culham air shower experiment which was run by the Harwell group in England arrived in Australia earlier during the year and has now been integrated into the former air shower experiment in Sydney. The American Air Force in Texas has recently flown £5,000's worth of nuclear emulsion for the emulsion group to a height of 126,000 ft. Balloons of 3,000,000 cu. ft. capacity were used. The flights were made gratis. The emulsion is to be processed in Chicago using the large developing facilities available in Prof. Marcel Schein's group.

The Wills Thermonuclear Department is a new one and has been made possible by a gift of £A.50,000 from W. D. and H. O. Wills (Australia) Ltd. in Sydney. The gift will be used for providing some of the initial research equipment required by the Department. The creation of a thermonuclear department in the School is in keeping with recent developments overseas in the thermonuclear field, where there has been a marked tendency for the research effort to swing back from large-scale engineering attacks to work of a more fundamental nature and on a smaller scale. It will also allow Australia to partake from an early stage in this exciting field of research.

The appointment of Prof. C. N. Watson Munro, chief scientist of the Australian Atomic Energy Commission, to the chair of thermonuclear physics is in keeping with his experience in the nuclear field. He was one of the pioneers in the nuclear reactor field and it is his wish now to try to pioneer some of the work in the thermonuclear field.

The Chatterton Astronomy Department will have two groups. One group will use a 40-in. reflecting telescope with auxiliary photoelectric equipment to consider problems in astronomy, the interpretation of which relies heavily upon physics. The contract for the instrument will be placed during 1960 and the instrument should be installed during 1961. Total initial cost of the project will be around £A.100,000. The site for the instrument has still to be determined, but is highly likely to be situated at a joint Mount Stromlo–Sydney School of Physics field station. A survey for the site is at present being carried out by the Mount Stromlo group.

The second group will in the first instance be concerned with work using an intensity interferometer (at a later date a general interferometry group will be built up). This instrument, the first of its kind to be built, is based upon the work of two British scientists, Mr. Hanbury Brown of the University of Manchester and Dr. R. Q. Twiss, who is a member of staff of the Chatterton Astronomy Department. The instrument will be used for measuring the temperature and diameter of stars—both very important quantities in astrophysics.

The contract for the instrument has been let in England and the interferometer will arrive in Australia towards the end of 1960. The site for the field station at which the instrument will be erected is likely to be at Narrabri, New South Wales—about 370 miles north-west of Sydney. Initial cost of the project will be in the neighbourhood of £A.175,000. Initial funds for work in the Astronomy Department were provided by a grant of £A.50,000 from Mr. Stan Chatterton, a prominent member of the Nuclear Research Foundation within the University of Sydney.

It will be noted that the five new departments within the School of Physics form an integrated research effort. The work of all the departments is closely linked and has been chosen with that purpose in mind. It should allow Australia to make an ever-increasing contribution to furthering new knowledge in these fields.

The Nuclear Research Foundation within the University of Sydney has been responsible for the provision of the major funds for the research effort in the School of Physics. A sum approaching £A.1,000,000 has been raised in various ways during the past six years.

OBITUARY

Dr. H. H. Potter

The death is announced of Dr. Harold Herbert Potter, lecturer in physics in the University of Bristol from 1924 until his retirement last summer owing to ill-health. Dr. Potter was a graduate of Bristol, who spent four years at King's College, London, as the holder of an 1851 Exhibition before returning to Bristol. At King's College, under O. W. Richardson, he carried out accurate measurements on the proportionality between mass and weight, and showed that the ratio is independent of the nature of the material within an experimental error of fifteen parts in a million.

To workers in the field of magnetism, Potter was well known for his investigations in Bristol in the field of ferromagnetics, both in collaboration with Sucksmith and independently between the two World Wars. This work included specific heat measurements through the Curie point; the magnetic properties of single crystals and their correlation with crystal structure, including Heusler and other magnetic alloys; and the electrical resistivity of nickel and iron from 20° to 1,200° K.

As an experimenter, Potter's work was careful, reliable and important in its period; it will also stand the test of time. In addition, he was a keen, effective and popular teacher of undergraduates.

He leaves a widow but no children.

NEWS and VIEWS

Chemistry at Bedford College, London: Prof. E. E. Turner, F.R.S.

THE retirement is announced of Prof. E. E. Turner, head of the Department of Chemistry at Bedford College, University of London. Prof. Turner received his early education in the Coopers' Company School, in Queen Mary College, and in Sidney Sussex College. He was assistant (chemical warfare research) to Sir William Pope, and he also served in the Research Department, Royal Arsenal, Woolwich. His first teaching appointments were in the University of Sydney and in Queen Mary College. Later, he was reader (1928–44) and professor (since 1944) at Bedford College, University of London. He is a Fellow of Queen Mary College, Freeman of the City of London, and Liveryman of the Coopers Company. He was elected to the fellowship of the Royal Society in 1939. Prof. Turner has made most distinguished contributions to research in organic chemistry, including especially stereochemical problems, such as molecular dissymmetry dependent on restricted rotation about a single bond in diphenyl and other compounds, asymmetric synthesis and transformation. He is joint author (with Margaret M. Harris) of a well-known text-book of organic chemistry. Prof. Turner's services to chemistry in many fields are far too numerous to mention; to give but one example, his colleagues in the University of London recall with pleasure and gratitude his many years of service on the Board of Studies in Chemistry, first as secretary and later as chairman.

Prof. P. B. D. de la Mare

DR. P. B. D. DE LA MARE, who is to succeed Prof. Turner, was educated at the Hamilton High School and Victoria University College, New Zealand. His interest and flair for research in physical–organic chemistry was first shown in important papers, in collaboration with Prof. P. W. Robertson, on the addition of halogens to olefins. Following his degree work at Wellington, he was directed for national service to the New Zealand Government Department of Agriculture, where he worked on problems of nutrition and the chemistry of lipids with Dr. F. B. Shorland. During 1946–48 he held a University of New Zealand fellowship at University College, London, and he was awarded the Ramsay Memorial Medal in 1948. Since that time he has been a member of staff of University College, where he is at present a reader in chemistry. He has made most notable contributions to the study of addition, substitution and re-arrangement processes in organic chemistry. He is a co-editor, with Dr. W. Klyne, of "Progress in Stereochemistry", vol. 2, and a co-author, with Dr. J. H. Ridd, of "Aromatic Substitution—Nitration and Halogenation". He is a member of the Publications Committee of the Chemical Society and of the Joint Library Committee. It is evident that the established traditions at Bedford College will continue to grow under Dr. de la Mare's direction.

Pierre Joseph van Beneden (1809–94)

THE distinguished Belgian zoologist, parasitologist and palæontologist, Pierre Joseph van Beneden, was born at Malines 150 years ago on December 19, 1809. After graduating M.D. at Louvain, he abandoned medical practice for natural history, but all his life maintained a keen interest in those branches of medicine which are aided by the study of the lower organisms. In 1831 he was appointed conservator of the Natural History Museum in Louvain, and in 1835 professor of zoology at the University of Ghent. In the following year he was elected to the corresponding chair at Louvain, which he occupied until his death. In 1843 he established at his own expense one of the earliest laboratories of marine zoology and aquaria. Author of some 200 brochures, his best-known works are "Zoologie Médicale" (1859) and "Ostéographie des Cétacées Vivants et Fossiles" (1868–80), both written in co-operation with Paul Gervais, and "Les Commensaux et les Parasites dans le Règne Animal" (1875; English translation, "Animal Parasites and Messmates", 4th ed., 1889). He made original contributions to scientific knowledge of the development of polypi and of worms, both intestinal and vesical, was the first to distinguish between 'commensalisme' and 'mutualisme', and enriched the catalogue of cetaceous fossils by several new species. Recipient of many honours, he was a foreign member of the Royal Society and of the Linnæan, Geological and Zoological Societies of London, and in 1881 served as president of the Royal Belgian Academy. In 1886 he was created Grand Officer of the Order of Leopold. A modest and kindly man, he died at Louvain on January 8, 1894. His fame has been eclipsed by that of his son, Édouard van Beneden (1846–1910), the embryologist.

Astronomy for the Layman

IN a series of articles in the *Illustrated London News* early in 1959, Dr. R. A. Lyttleton described the astronomical universe starting at the Earth and working outwards. These articles proved of great interest, and consequently the *Illustrated London News* is publishing a second series of astronomical articles; this time they are written by Prof. H. Bondi, of King's College, London. The first appeared on October 31, and the remaining eleven articles are following at weekly intervals. As before, the articles are accompanied by photographs and by illustrations drawn by Mr. G. H. Davis, the special artist of the *Illustrated London News*. Prof. Bondi started with the most distant objects observed, and will work inwards in later articles. After some preliminary remarks on the philosophical basis of science he discusses the expansion of the universe. His second article discusses the famous paradox of Olbers (Why is it dark at night?), and this is followed in the third and fourth articles by an account of the evolutionary and steady-state theories of cosmology and of possible observational tests of these theories. In subsequent articles Prof. Bondi will discuss the stars, interstellar and interplanetary space and gravitational problems in the solar system, ending with the motion and magnetism of the Earth.

Management in India

IN her five-year plan, India is trying democratically to meet the desperate needs of her peoples for better living conditions. Industrialization is proceeding rapidly, and it is encouraging to read how leaders of

industry in India are not only trying to avoid the mistakes made by Western countries during their industrial revolution but are also seeking to utilize the best practices of the best industrial organizations in Western countries. The Ahmedabad Textile Industry's Research Association has attracted interest for the attention it is paying to the industrial application of research, the use of work study, and the training of technologists and technicians. Its latest venture is the holding of a conference deriving its inspiration from H.R.H. the Duke of Edinburgh's Study Conference on the Human Problems of Industrial Communities within the Commonwealth and Empire. Many of the subjects selected for study were the same as those arranged at the Duke of Edinburgh's conference at Oxford. It was particularly appropriate that the Duke was able to open the conference, while another distinguished speaker was Sir Alexander Fleck. The proceedings of the Conference (Ahmedabad Textile Industry's Research Association. Proceedings of the Fifth Management Conference, January 28, 29 and 30, 1959. Pp. ix+93. Ahmedabad: Ahmedabad Textile Industry's Research Association, 1959) have now been published.

A Giant Plesiosaur

About sixty years ago a fossilized piece of the mandible of a large skull, with three broken teeth preserved on each side, was found in rocks of the Lower Cretaceous near Hughenden, Queensland. The specimen, which is in the collection of the Queensland Museum, is about 8 in. long and 5½ in. in height and breadth. The imperfect teeth were estimated to have been originally at least 10 in. long. The fragment was tentatively identified as a portion of a mandible of an ichthyosaurus. In 1923, the late Mr. Heber Longman, then director of the Queensland Museum, decided that it belonged to a new type of plesiosaur, and the animal was named *Kronosaurus queenslandicus*. In 1931, Mr. William Schevill, of the Museum of Comparative Zoology at Harvard College, U.S.A., while passing through Army Downs, North Queensland, noticed a number of fossil bones partly showing in the Lower Cretaceous rocks. Four and a half tons of rock was blasted out with dynamite and packed and despatched to the Museum at Harvard. After its arrival, Arnold Lewis, a Museum assistant, attempted to free the bones from the hard rock. About six months ago this work was successfully completed, and the skeleton was put together under the direction of the Museum director, Dr. Alfred S. Romer. The skeleton is 42 ft. in length. It has a triangular-shaped skull, 9 ft. long, with eighty spiked teeth each up to 8 in. in length. The characters of the teeth and mandible are identical with those of the mandible fragment recorded by Longman in 1923 (*Austral. Mus. Mag.*, **13**, No. 2; June 15, 1959).

Syncarid Crustacea

Until recently only one syncarid was known from the Australian mainland (*Austral. Mus. Mag.*, **13**, No. 2; June 15, 1959). This is *Koonunga*, which was described in 1907. Originally it had been found at Ringwood, now an outer suburb of Melbourne, and the stream in which it lived was long ago converted to a storm-water drain. In 1930 it was reported that *Koonunga* was extinct. During the past few years it has turned up in a variety of places, and is now known to occur sporadically across the southern part of Victoria. *Koonunga* is about one-third of an inch long, brownish in colour, and has a slender, almost cylindrical body. Its eyes are very small, and, unlike those of *Anaspides* and shrimps, are not stalked. It can swim actively, using its legs and abdominal appendages, but is more often found running rapidly over the bottom of pools and streams. This method of locomotion is so distinctive that *Koonunga* cannot be mistaken for any other aquatic animal. It relies on its agility and on its capacity for burrowing into mud to escape from enemies, for it is helpless against predatory aquatic insects.

Koonunga lives in small permanent or semi-permanent swamps and in pools in streams which flow vigorously only after heavy rains. Since many of these places are apt to become dry during the summer, and since the adults cannot withstand desiccation, it had been supposed that *Koonunga*, like so many other freshwater crustaceans, would lay drought-resistant eggs. It is now known, however, that the breeding season is during winter. The eggs, which are inserted singly or in small batches into decaying plants, develop over a period of about two months and hatch in the spring. It appeared that *Koonunga* could survive drought only by descending underground; they have been obtained from pits 2–3 ft. deep in the beds of dried-out streams. *Koonunga* can burrow swiftly into soft mud, but would not seem capable of forcing its way through heavy subsoil. The indications are that, in making its descent, it uses the burrows of freshwater crayfish. These crayfish are known to follow the water-table down to a depth of 6 ft. or more, and could thus provide a safe refuge for *Koonunga* during periods of drought. In some places *Koonunga* must remain underground continuously for six months or more. From this mode of life it would seem only a short step to a wholly subterranean existence, and work in Victoria over the past few years indicates the presence of a rich underground fauna of syncarids. Already, four new species have been found. They lack eyes, and have suffered a general reduction in pigmentation so that they have a translucent, white appearance. The largest species is nearly half an inch long, the smallest only one-sixth of an inch. Though these syncarids are typically subterranean in habit, they may, in special circumstances, live in surface water, and this is where they have been found. Pools are advantageous to syncarids in that few other aquatic animals live in them and there is little competition. The survival of *Anaspides* almost unchanged since Palæozoic times has been attributed to its living in a stable environment and to its freedom from predation. Conditions on the mainland are less stable. *Koonunga* and its allies would seem to owe their survival to their close association with the freshwater and land crayfishes.

The Liverpool Geological Society

The centenary of the Liverpool Geological Society will be celebrated during January 1–3 by a three-day meeting devoted to recent advances in geology. At this meeting, run jointly with the eighth Inter-University Geological Congress in the University of Liverpool, the following addresses will be delivered: Dr. W. S. Mackenzie, on "Experimental Petrology"; Prof. S. I. Tomkeieff, on "The Progress of Geology in the U.S.S.R."; Dr. G. Voll, on "Petrofabrics and Small-scale Structures"; Prof. C. F. Davidson, on

"Aspects of Radiogeology"; Dr. R. W. Girdler (in the absence of Dr. R. G. Mason), on "Geophysical Researches on the Sea Floor"; Dr. J. A. Clegg, on "Palæomagnetism"; Prof. W. C. Krumbein, on "Numerical Data in Geology"; Dr. L. M. J. U. van Straaten, on "Deltaic Sedimentation"; Dr. R. H. Cummings, on "Micropalæontology"; Dr. J. S. Weiner (in the absence of Prof. T. S. Westoll), on "Sequences in Hominoid Evolution". The address by Prof. T. S. Westoll on "Vertebrate Palæontology", though not given at the meeting, will be published with the other addresses in January in the centenary issue of the *Liverpool and Manchester Geological Journal*. Dr. Clegg's and Dr. Weiner's addresses will be published in abstract. Details of the meeting can be obtained from R. G. C. Bathurst, Department of Geology, University of Liverpool.

The Night Sky in January

FULL moon occurs on Jan. 13d. 23h. 51m. U.T. and new moon on Jan. 28d. 06h. 16m. The following conjunctions with the Moon take place: Jan. 10d. 09h., Aldebaran 0·7° S.; Jan. 25d. 08h., Jupiter 5° S.; Jan. 25d. 15h., Venus 4° S. In addition to these conjunctions with the Moon, Venus is in conjunction with Antares on Jan. 9d. 04h., Venus being 7° N., Mercury with Saturn on Jan. 11d. 04h., Mercury being 1·8° S., Venus with Jupiter on Jan. 21d. 11h., Venus being 1·1° N., and Mars with Saturn on Jan. 31d. 11h., Mars being 1·2° S. Mercury is too close to the Sun for observation. Venus is a morning star, visible low in the south-east before sunrise. It rises at 4h. 40m. and 5h. 45m. on January 1 and 31, respectively. Its stellar magnitude is −3·6; its distance increases during the month from 98 to 116 million miles and the visible portion of the apparent disk increases from 0·714 to 0·809. Mars is badly placed for observation, rising about an hour before the Sun. Jupiter is a morning star, rising at 6h. 30m., 5h. 50m. and 5h. 00m. on January 1, 15 and 31, respectively. It may be seen low in the south-east before sunrise; its stellar magnitude is −1·4. Saturn is too close to the Sun for observation. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Jan. 10d. 17h. 39·7m., 318 *B*. Tau. (*D*); Jan. 11d. 17h. 41·9m., 130 Tau. (*D*); Jan. 12d. 20h. 12·7m., 26 Gem. (*D*). *D* refers to disappearance. The Quadrantid Meteors are active on January 3–4, with a predicted maximum at Jan. 4d. 05h. The radiant is near R.A. 15h. 28m., Dec. + 50°, and conditions are favourable for observation. The Earth is at perihelion on January 4, its distance from the Sun being 91·4 million miles.

University News:

Birmingham

DR. A. P. D. THOMSON, senior lecturer in anatomy, has been appointed acting director of the Department of Anatomy as from January 1. The following appointments have been made: Dr. D. S. Urch, lecturer in chemistry; J. C. Maltbaek, lecturer in mechanical engineering; G. C-Y. Hu, lecturer in highway and traffic engineering; G. H. J. Bennett, lecturer in industrial metallurgy. Dr. C. G. Wall, lecturer in chemical engineering; R. H. Jones, assistant lecturer in civil engineering; P. J. Waterton, staff Fellow in physics; Dr. V. Knapp, research Fellow in nuclear physics; Dr. M. Gage, research Fellow in geology; M. H. B. Hayes, I.C.I. Fellow in chemistry; Dr. J. P. Simons, Fellow in chemistry; D. W. Thomas, National Coal Board research Fellow in physical chemistry.

Glasgow

THE Minister for Science, the Right Hon. the Viscount Hailsham, was elected Rector of the University on October 26.

The new Engineering Building was inaugurated by the Right Hon. the Viscount Montgomery of Alamein on November 4.

The following have been appointed to lectureships: R. E. Azuma and W. R. Hogg, experimental and theoretical physics; Helen S. Dunsmore and G. S. Harris, chemistry; W. Graham Jardine, geology; and J. Leech, computing laboratory; and the following to research fellowships: A. Bhati, A. C. Day, W. J. McMaster and W. Parker, chemistry; J. D. Roycroft, solid state physics; and Wm. C. Russell, virology (first Lock fellowship).

For the present session, 392 new students (302 men and 90 women) have been admitted to the Science Departments, compared with 372 in 1958. The total of matriculated students in the University as a whole now stands at 6,566, compared with 6,330.

Southampton

THE following appointments have been announced: Dr. E. M. Wilson, senior lecturer and director of computation; Mr. P. C. Parks, lecturer in aeronautical engineering; Mr. G. G. Bloodworth, lecturer in electronics; Mr. J. F. Barrett, De Havilland research Fellow in aeronautical engineering; Mr. M. T. Thew, Vokes research Fellow in mechanical engineering.

Announcements

THE fifth Levinstein Memorial Lecture of the Society of Chemical Industry will be delivered by Mr. L. H. Williams on February 5. He will speak on "The Future of Chemicals from Petroleum" and the lecture, which is open to the public, will be given in the Large Chemistry Theatre, University of Manchester, at 6.30 p.m.

THE British Welding Research Association and the Engineering Department, the University of Cambridge, are jointly organizing a symposium under the title of "Fatigue of Welded Structures". This is to be held in the Department of Engineering at the University of Cambridge during March 29–April 1. Further information can be obtained from the British Welding Research Association, Abington Hall, Abington, Cambs.

A POSTGRADUATE course of nine lectures on "Newer Developments in the Chemistry of the Non-Metals" will be given at Kingston Technical College during January 27–March 23. The fee for the course is £1 2*s*. 6*d*. Further information can be obtained from the Principal, Kingston Technical College, Fassett Road, Kingston-upon-Thames.

KODAK, LTD., are offering up to six annual scholarships to be known as "Kodak Scholarships in Colour Photography". Successful candidates will be given about three months specialized instruction in the laboratories and studios of the Eastman Co. in Rochester, New York. Approved expenses will be paid. Further information can be obtained from the Secretary, Kodak, Ltd., Kingsway, London, W.C.2.

BRITISH AGRICULTURAL HISTORY SOCIETY

THE British Agricultural History Society held its annual winter conference, in co-operation with the Association of Agriculture, at the Institute of Education, University of London, on December 5, under the chairmanship of its newly elected president, Sir Keith Murray, who was welcomed with acclaim. Sir James Scott Watson retired this year after having been president of the Society since its inception in 1953.

Unfortunately, sudden illness prevented Mr. W. B. Mercer from attending to give his proposed lecture on the "Development of Agricultural Education and Advisory Services". This hiatus in the programme was ably filled by Mr. T. W. Fletcher, of the Department of Agricultural Economics, University of Manchester, with a paper on "Lancashire Livestock Farming in the Great Depression".

Mr. Fletcher, whose views on the farming history, especially of Lancashire, in the past three decades of the nineteenth century are somewhat revolutionary, showed that the livestock farmers of Lancashire did not, in fact, suffer in the depression. On the contrary, their standards of output, purchase of imported concentrates and other feeding stuffs, as well as their incomes, improved. The reasons for this were not far to seek. The local demand, both number of urban mouths and size of urban incomes, was growing. Consequently, the livestock farmers had a market, so to speak, on their doorstep for all the milk, butter, cheese, beef, mutton and pork that they could produce. On the east side of the county, too, there was a large demand from the West Riding industrial district. In such circumstances, it is not surprising to learn that rents were well paid and stable, and that relations between landowners and tenants were amicable.

The afternoon session was opened by Mr. O. R. McGregor, reader in social institutions, University of London, with a paper, "Free Trade in Land in the Victorian Period". Mr. McGregor argued, in a way that would certainly have pleased Maitland and Vinogradoff, that in any event for the nineteenth century the study of the social institutions surrounding farming was more important than the study of the development of technical changes. With wide reference to contemporary dialectic he emphasized the conflict between the rising class of urban industrialists and of the small number of great landowners (not more than about a thousand) who were striving by all means to retain their dominance of the political scene, and the prestige and patronage, for example, in Army and Church, that derived from it. He emphasized that the landowners, who used every possible device to prevent the break-up of estates—primogeniture, entail and settlements—regarded the land not so much as a rent- or food-producing commodity, but as a possession of social value. This outlook coloured their relation with their tenants, whom they wished to keep in mercy as tenants-at-will, and their attitude towards the increasingly wealthy and pugnacious middle classes who demanded 'free trade in land'. It was significant that so many members of the House of Commons before 1870 were landowners and land agents, not to speak of the House of Lords. This lecture certainly provided a new approach to the problems of farming history in the nineteenth century.

Finally, Miss Edith Whetham, Gilbey lecturer in the history and economics of agriculture, University of Cambridge, discussed "Cambridgeshire Tithe Maps, 1836–1890". She said that the county was backward in 1836 and that the tithe maps therefore gave a fine illustration of the change from open to enclosed fields in many parishes. She did not develop the subject at length, preferring to allow the documents exhibited, and the photostats passed around, to confirm it for her.

G. E. Fussell

NATIONAL RESEARCH COUNCIL OF CANADA

STRESS has been laid on a recent report on the support given to postgraduate research in the universities of Canada, for which some 34 million dollars have been provided by the National Research Council of Canada since 1917*. In the current year, 5·9 million dollars were provided for this purpose by way of 681 grants and 435 scholarships, fellowships and associateships, and expenditure is expected to reach 8·2 million dollars in 1959–60. The Council itself employs a scientific research staff of 613 (including 149 postdoctorate Fellows), 883 technicians and 887 general service and administrative staff. Much of the work of the Division of Applied Biology is in the applied field, and its statistical studies of short-term protein variability in cargoes of Northern wheat exported from Vancouver were expanded (in co-operation with the Grain Research Laboratory Board of Grain Commissioners) to permit accounting for this variability as the resultant of random differences between successive car loads from the same shipping point, of weekly variations in the geographical pattern of car-loads, and of mixing incidental to terminal limeing, handling and loading. A study of the effect of exposure on new-born caribou indicated that caribou calves are quite sensitive to cold and wind, to which they respond by a marked increase in metabolism. Investigations are also in progress on the fundamental properties of the hemicelluloses of common pulping woods, designed to assist pulp-makers in the production and use of semi-chemical pulp, but the report emphasizes that the biological sciences occupy an unfavourable position in the overall Canadian scientific effort.

At the Atlantic Regional Laboratory, Halifax, a chelating chemical as a dip before frying was found to

* Forty-Second Annual Report of the National Research Council of Canada, 1958–59, including the Annual Report of Canadian Patents and Development Limited. Pp. 26. (Ottawa: Queen's Printer, 1959.)

prevent 'greying' in the production of partly precooked French fried potatoes, due to an abnormally high content of iron in the environment, while at the Prairie Regional Laboratory, Saskatoon, gas–liquid chromatography has been used for the quantitative determination of fatty acids in oils and fats, and a new type of foam breaker developed which operates on the principle of rapid acceleration of the foam through a nozzle, using only pressure from air charged into the fomenter. The work of the Division of Applied Chemistry has gradually changed in recent years in an effort to obtain broader information in specific fields so that *ad hoc* problems have received less attention. Much of its work is related to petroleum and corrosion chemistry. In the Division of Pure Chemistry, which is organized in thirteen sections, outstanding achievements of the year were the determination of the total structure of aconitine, the establishment of characteristic zone patterns for each type of functional group, following the determination of the infra-red spectra of many steroids, and the development of the new technique, known as nuclear magnetic resonance, in which the nucleus within a molecule is used as a 'probe' to investigate details of molecular structure.

The Division of Applied Physics has produced a new kind of plotting instrument for making maps from air photographs, and the Division is also investigating the suitability of using for the measurement of time an invariable frequency of vibration emitted by some atom or molecule, and observations over six months have shown promising agreement of the cæsium 'clock' with the one at the National Physical Laboratory, Teddington. Much cosmic-ray work was carried out for the International Geophysical Year by the Division of Pure Physics, and micro-wave technique was used to determine precisely the distances between the atoms in molecules of acetone, propiolic aldehyde, vinyl cyanide, nitric acid, methyldifluorsilane and formamide, while a detailed theory has been developed which appears to be in excellent agreement with experimental data on the multiple scattering of charged particles. The thermoelectric powers of copper and silver, among others, at temperatures much below 1° absolute have been found to be extraordinarily high, higher by orders of magnitude than present-day theory predicted.

In October 1958 the new Fire Research Building was officially opened as the final part of the Building Research Centre, and in its work of keeping the National Building Code of Canada up to date the Division of Building Research has undertaken studies of the actual loads on structures due to floor loadings, wind and snow. In the Division of Mechanical Engineering, emphasis on projects relating to improvements in transport in Canada continued, including harbour improvements, winter navigation by water, rail transport and various aspects of the evolution of new aircraft capable of vertical take-off and landing.

About half the programme of the Radio and Electrical Engineering Division was concerned with defence. Techniques were developed for producing and measuring extremely low pressures in gases, and the Division co-operated with the National Aeronautical Establishment in developing a small radio transmitting beacon which is flung clear from the tail of the aircraft in the event of disaster. In co-operation with the Marine Services Branch of the Department of Transport, electric circuits are being applied to marine navigational devices, often for isolated locations.

DYNAMIC AND STATIC DEFORMATION OF ARMCO IRON

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Canadian Armament Research and Development Establishment, Quebec

A RECENT article on the above subject[1] showed that the mechanical properties of Armco iron were slightly lower after a previous amount of dynamic prestrain than after the same amount of static deformation. This finding confirmed the results of Campbell and Duby[2] but was contrary to those of Harris and White[3]. The object of the present article is to give further results concerning the effects of variables such as amount of prestrain and temperature of deformation.

The same method of dropping a weight on a specimen 0·75 in. diameter and 1·5 in. long placed on a large solid anvil was used. By varying the height of drop, the velocity of impact and thus the amount of deformation were changed. Other specimens were then deformed statically by the same amounts obtained during dynamic impact. After deformation, the specimens were cut in two longitudinally, with one half hardness tested before ageing and the other after ageing. The results are shown in Fig. 1. The hardness was found to increase with amount of deformation for both static and dynamic deformation and the rate of increase in hardness was about the same for the two methods of prestraining. The increase in hardness, however, is less for the dynamically than for the statically deformed specimens, for the whole range of deformation used, and this difference is approximately constant for that range of deformations.

After ageing the difference in hardness is again approximately constant for both statically and dynamically deformed specimens. If, on the other hand, one considers the difference in hardness between the aged and unaged condition for both static and dynamic prestrain, it will be seen that this difference increases with higher amounts of deformation. This observation confirms established beliefs that the tensile strength of iron after strain-ageing increases with the amount of static prestrain. It is seen here that the same phenomenon is observed with dynamic prestrain if we assume that tensile strength is proportional to hardness. Hundy[4] has suggested that this effect could be ascribed to the formation of discrete submicroscopic carbide or nitride precipitates at dislocation junctures.

The fact that hardness and other mechanical properties are lower after dynamic than after static prestrain was corroborated by compression and

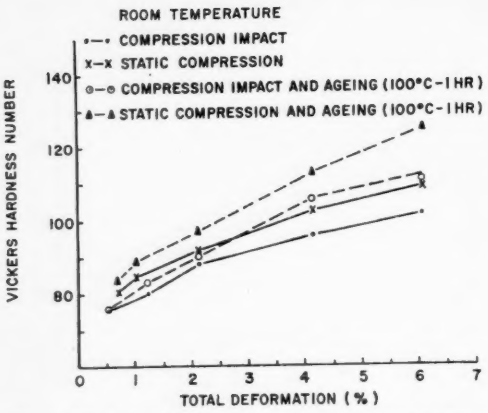

Fig. 1. Effect of total static or dynamic deformation on hardness before and after ageing

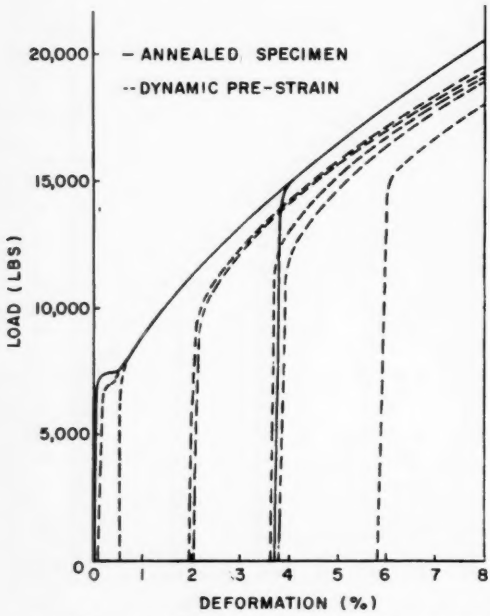

Fig. 2. Static load-deformation curves in compression showing softening of previously impacted specimens

tensile test results as shown in Figs. 2 and 3. The compression curves for specimens in the unaged condition all lie below the compression curves obtained for specimens deformed after comparable amounts of static prestrain. The same result was observed for statically and dynamically deformed specimens in the aged condition.

When the temperature is varied for a given height of drop, the same trends are observed, as seen in Fig. 4, except for the lowest temperature of testing (— 196° C.) where the results are about the same as those for the temperature of (— 75° C.). But again the hardness obtained after dynamic deformation is lower than that after static deformation, at all temperatures tested, both in the unaged and aged condition.

It can be assumed, as has been proposed by Campbell and Duby[3], that dynamic impact will activate a larger number of dislocations than static deformation because of the much higher stresses applied during the short-time dynamic loading. The larger number and more uniform distribution of free dislocations when compared with the same amount of static prestrain will permit the metal to deform more easily upon subsequent reloading. Consequently, the static mechanical properties of a dynamically prestrained specimen should be lower than that of a statically prestrained specimen as is seen to be the case from hardness, compression and tensile tests.

In two other series of tests, the deformation was restrained to a constant value while the temperature and the height of drop were varied. This restriction of the amount of deformation was accomplished by inserting an impactor arrester around the specimen, so that the impactor would be stopped at a given height and deform specimens by constant amounts independently of height of drop and temperature. The results seen in Fig. 5 show that the hardness of the dynamically deformed specimens, especially for the unaged condition, does not vary appreciably with height of drop, that is, velocity of impact, or with temperature for a constant deformation. This would thus imply that in the case of dynamic deformation the work-hardening is independent of velocity and temperature of deformation in the ranges used and that the hardness is a function mainly of the amount of deformation.

A similar conclusion had been reached previously by Campbell and Maiden[4], who prestrained two specimens by the same total amount of deformation, one by impacting twice at — 84° C. at 503 in./sec., the other by impacting once at room temperature at 460 in./sec. No significant difference was observed in the stress-strain curve, indicating that the shape

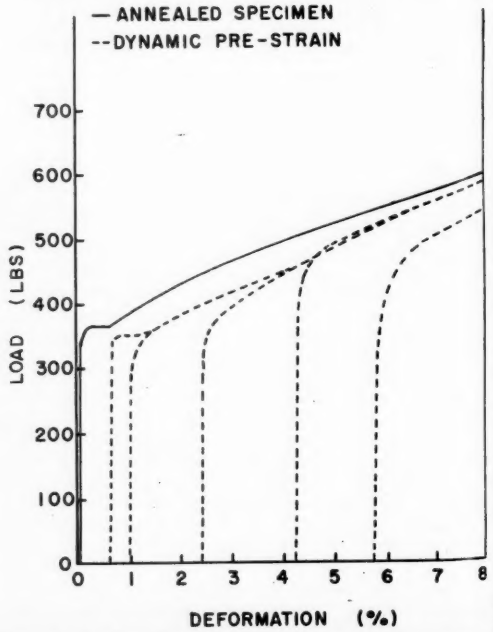

Fig. 3. Static tensile test results on annealed and dynamically prestrained specimens

of the curve was dependent mainly on the amount of deformation and not on the temperature, velocity or even number of impacts during deformation.

On a dislocation basis, it would also appear that the number of dislocations produced is independent of velocity of deformation and of temperature of tests but would depend mainly on the amount of deformation during the impact as was discussed previously[5]. The increase in hardness after ageing these specimens was also independent of height of drop and temperature.

It was thought of interest to determine the effect of temperature of static prestraining on the mechanical properties during subsequent static deformation at room temperature. Fig. 6 shows that low-temperature static prestraining has an effect similar to that of a dynamic prestrain in lowering the stress-strain curve below that obtained after static prestrain at room temperature.

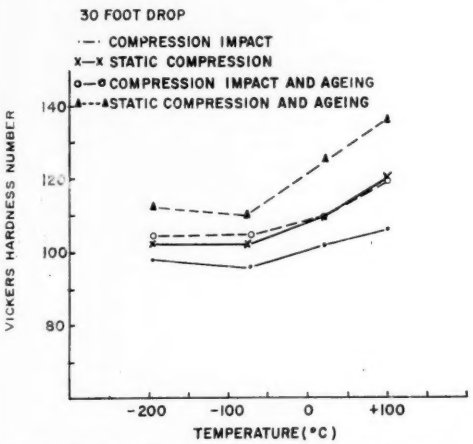

Fig. 4. Effect of temperature of deformation on hardness of statically and dynamically deformed specimens

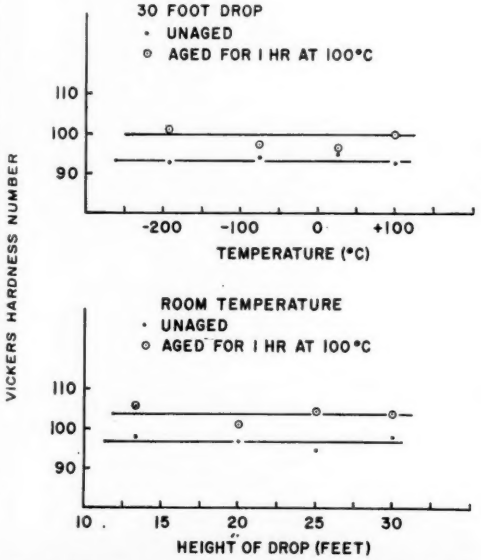

Fig. 5. Effect of temperature and velocity of deformation in 'restrained deformation' tests on resultant hardness

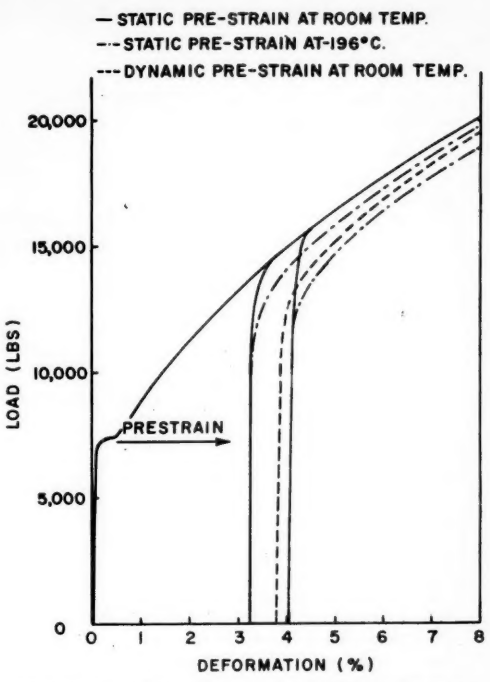

Fig. 6. Static load-deformation curves in compression for unaged, statically and dynamically prestrained specimens, illustrating the equivalence of strain-rate and temperature

This would mean that Campbell and Maiden's observation[5] that there was little difference in the stress-strain curves for the two specimens impacted at different temperatures and our observation of Fig. 5 where hardness is independent of temperature of deformation would only apply for dynamic prestrain. In the case of static prestrain, prestraining at low temperature will have an effect on the subsequent static curve, equivalent to that of a dynamic pre-deformation. By extending Campbell and Duby's theory one could say that the activation of a larger number of dislocation sources taking place under impact also takes place during low-temperature static deformation. At low temperature, the stress that must be applied to produce deformation is much higher than at room temperature (the 0·2 per cent proof stress being 3½ times higher than at room temperature for the Armco iron tested), permitting a larger number of dislocation sources to be released if we assume that dislocation sources can be released over a range of activation energies. On this basis, one would expect diffuse slip during static deformation at low temperature, and this was observed to be so in the course of metallographic examination of specimens. In a very recent paper Maiden and Campbell[6] have in fact observed the presence of diffuse slip during static deformation at low temperatures, and the equivalence of high strain-rates and low temperatures on mechanical properties of low carbon steels.

This article corroborates the foregoing observations on iron and extend them to the case of prestraining under these conditions.

It thus seems that the equivalence of effects of high strain-rates and low temperatures does not apply only during deformation as is already known, but also that their effects during prestraining will influence the behaviour of iron in the same direction during subsequent straining.

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AGGREGATION OF AN ACRIDINE DYE ON NATIVE AND DENATURED DEOXYRIBONUCLEATES

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ACRIDINE orange has been shown[1] to form aggregates or 'stacks' when bound to polyanions. The strength of the dye–dye interaction depends upon the polyion used, and is, in general, greater with flexible than with rigid polymers. Since deoxyribonucleic acid undergoes a transition from a rigid, two-stranded helix to a flexible, random coil upon heating[2], it might be expected that an increase in dye–dye interaction would accompany this transition. We wish to report the observation of such an increase in dye-stacking tendency accompanying the heat denaturation of deoxyribonucleic acid.

Calf thymus deoxyribonucleic acid ('Sigma') was denatured by slowly heating a solution (2 mgm./ml., M/1,000 cacodylate buffer pH = 6·7) and removing aliquots after 10 min. at specified temperatures. The helix → coil transition was followed by the drop in viscosity and the increase in ultra-violet absorption at 260 mμ (Table 1 and Fig. 1), and because of the low ionic strength used, occurred at relatively low temperatures (T_m = 62°).

Spectrophotometric titrations of acridine orange with native and denatured deoxyribonucleic acids were carried out as previously described[1]. Sharp end-points were obtained at 1 deoxyribonucleic acid phosphate per acridine orange ion (Fig. 2). The volume of deoxyribonucleic acid required to titrate a given amount of dye did not change with denaturation (Table 1), showing that no additional binding sites were produced by heating. The end-point of the titration therefore provides a method for determining the concentration of deoxyribonucleic acid which is convenient, independent of the extent of denaturation, and which requires only a small amount of deoxyribonucleic acid (about 2–20γ).

Sedimentation studies showed essentially complete binding of the dye at the 1 : 1 point under the conditions used ($1·6 \times 10^{-5}$ M acridine orange, 1×10^{-3} M cacodylate buffer, pH = 6·7). At the 1 : 1 point, therefore, each deoxyribonucleic acid binding site is occupied, and every dye molecule has a dye molecule on each adjacent site with which to interact. The spectrum of the dye in this 1 : 1 complex shows two bands, an α band or shoulder at 492 mμ which predominates in the free-acridine orange monomer spectrum, and a β band at 464 mμ which predominates in the free acridine orange dimer spectrum[1,3]. The relative heights of these two bands (β/α ratio) may therefore be considered to be a measure of the dimeric character of a dye molecule which is involved in the maximum number of dye–dye interactions possible on the particular polyanion. As can be seen in Table 1 and Fig. 1, this measure (β/α ratio) of dimeric character, or of stacking tendency, increases with denaturation, and further, the fractional increase in β/α follows closely the fractional changes in both viscosity and ultra-violet absorption.

When measured under the above conditions, the β/α ratios of 1 : 1 complexes of acridine orange with a number of different native deoxyribonucleic acid samples are all about 1·28 (Stone, A. L., and Bradley, D. F., unpublished results), so that a low β/α ratio of this magnitude may be used as one criterion for the 'nativeness' of deoxyribonucleic acid. However,

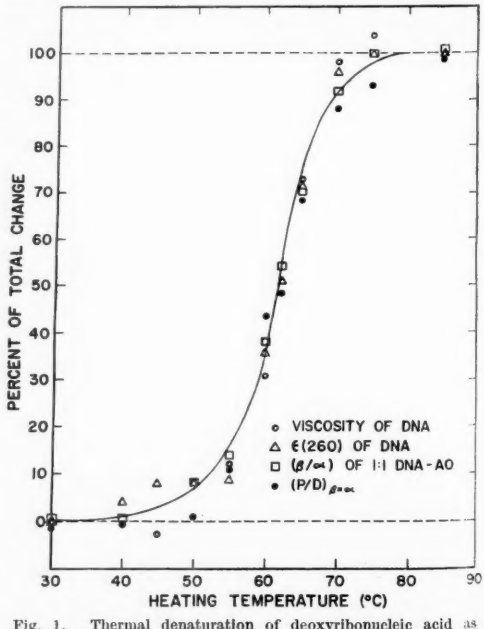

Fig. 1. Thermal denaturation of deoxyribonucleic acid as measured by changes in properties of deoxyribonucleic acid and deoxyribonucleic acid-bound acridine orange. Abscissa: temperatures to which deoxyribonucleic acid solutions were heated. Ordinate: fractional changes in viscosity and ultra-violet absorption of deoxyribonucleic acid, and of visible absorption of acridine orange bound to the heated deoxyribonucleic acid. For precise definition of quantities see legend to Table 1

Table 1. SUMMARY OF DATA ON THE HEAT DENATURATION OF DEOXYRIBONUCLEIC ACID

Sample	DNA viscosity [η]	%	Ultra-violet absorption E_{260}	%	ml. DNA/mole AO ($\times 10^{-8}$)	Dimeric character $(\beta/\alpha)_{1:1}$	%	Unstacking rate $dE/d(P/D)$	%	Equal α and β bands $(P/D)_{\beta=\alpha}$	%	Stacking coefficient K
Native CT–DNA			6,000		1·02	1·31		9,200		1·91		1·16 ± 0·14
CT, 30°	3,100	0	5,800	0	1·03	1·28	0	8,600	0	1·93	0	
CT, 40°			5,900	4	1·04	1·28	0	8,300	12	1·93	0	
CT, 45°	3,200	−3	6,000	8	1·04			8,300	12			
CT, 50°			6,000	8	1·05	1·31	8	8,700	−4	1·94	1	
CT, 55°	2,800	12	6,000	8	1·01	1·33	14	7,500	44	2·09	11	
CT, 60°	2,200	31	6,700	36	1·03	1·42	38	5,200	96	2·54	43	
CT, 62°			7,070	51		1·48	54	5,540	82	2·61	48	2·15 ± 0·07
CT, 65°	1,000	73	7,600	72	1·07	1·54	70	5,200	96	2·92	69	
CT, 70°	310	98	8,200	96	1·08	1·62	92	5,100	100	3·19	88	
CT, 75°	130	104	8,300	100	1·01	1·65	100	5,200	96	3·26	93	
CT, 85°	240	100	8,300	100	0·91	1·65	100	5,100	100	3·36	100	
Boiled CT–DNA			8,800		1·15	1·64		6,400		3·17		5·36 ± 2·44
SS–DNA	90		7,500			1·54		4,450		3·68		3·49 ± 0·48
S–RNA			7,750			1·57		3,260		4·82		3·27 ± 0·13
AT–RNA			7,890			1·72		4,960		4·90		3·22 ± 0·32

CT, calf thymus deoxyribonucleic acid (Sigma Chemical Co.); SS–DNA, salmon sperm deoxyribonucleic acid (California Foundation); S-RNA, soluble rat liver ribonucleic acid (S. Luborsky); AT–RNA, ascites tumour ribonucleic acid (J. Colter). [η] is η_{sp}/c. in cm.^{3}gm.$^{-1}$ of 0·004 per cent deoxyribonucleic acid solutions in 0·25 M sodium chloride measured at an average shear ($\bar{\beta}$) of 209 sec.$^{-1}$. E_{260} is the molar extinction of deoxyribonucleic acid at 260 mμ calculated using the deoxyribonucleic acid phosphate molarity obtained from the spectrophotometric titration with acridine orange. ml. DNA/mole AO is the volume of deoxyribonucleic acid required per mole acridine orange to reach the 1 : 1 point in the spectrophotometric titration with acridine orange (about $4·6 \times 10^{-8}$ mole). $(\beta/\alpha)_{1:1}$ is the ratio of extinction at 464 mμ to 492 mμ of the dye in the 1 : 1 complex. $dE/d(P/D)$ is the initial rate of change in E_{504} mμ with phosphate/dye ratio for $P/D > 1$. $(P/D)_{\beta=\alpha}$ is the ratio of deoxyribonucleic acid phosphates to acridine orange ions at which the extinctions at 464 and 492 are equal. K's are the stacking coefficients (see text) with their mean deviations. %'s are percentage changes in properties between 30 and 85°.

since the 1 : 1 complex is quite easily dissociated into free acridine orange and deoxyribonucleic acid by dilution or by added salts, resulting in an increased α (monomer) band and spuriously low β/α ratio, the conditions under which β/α is determined must be carefully controlled. For example, the complex may be 50 per cent dissociated ($\beta/\alpha = 1·0$) either by lowering the acridine orange concentration to $0·8 \times 10^{-5}$ M or by raising the buffer concentration to 4×10^{-3} M. This extreme sensitivity to salt concentration accounts for the failure of Steiner and Beers[1] to observe a 1 : 1 complex with native deoxyribonucleic acid in 0·06 M salt and $0·7 \times 10^{-6}$ M acridine orange. However, these authors do observe a 1 : 1 complex with boiled deoxyribonucleic acid under these same conditions, indicating that the dye–polymer interaction is increased by disruption of the ordered deoxyribonucleic acid structure. Kurnick[5] and Lawley[6] have also noted that heat denaturation and depolymerization affect the affinity of deoxyribonucleic acid for methyl green, rosaniline, and 5-aminoacridine.

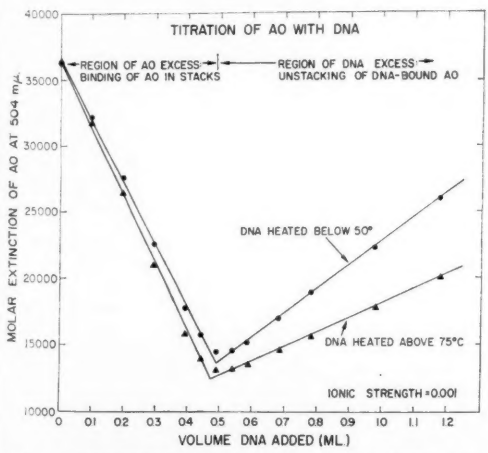

Fig. 2. Spectrophotometric titration of acridine orange with deoxyribonucleic acid. Molar extinctions of dye at 504 mμ calculated from volume-corrected optical densities. M/1,000 cacodylate buffer, pH 6·7. Deoxyribonucleic acid solutions about 0·04 mgm./ml. Upper curve, deoxyribonucleic acid solution (2 mgm./ml.) slowly heated to 50° C., aliquot removed after 10 min., cooled and diluted; moles acridine orange = $4·65 \times 10^{-8}$, molarity acridine orange at end-point = $1·61 \times 10^{-5}$; lower curve, same as above but heated to 75° C.; moles acridine orange = $4·60 \times 10^{-8}$, molarity acridine orange at end-point = $1·60 \times 10^{-5}$. Titrations carried out at 27–28° C.

The $T2$ phage deoxyribonucleic acid prepared by the phenol extraction method is unique in showing $\beta/\alpha = 1·0$ under all conditions, suggesting that either this deoxyribonucleic acid is more native than the others, or that stacking and/or binding is interfered with by the glucose groups or by residual bound spermine, etc. Despite such variations in its absolute numerical value, the fractional increase in β/α with denaturation follows closely the viscosity and ultraviolet absorption curves under a variety of experimental conditions.

On the deoxyribonucleic acid-excess side of the 1 : 1 point the bound acridine orange molecules begin to unstack as they distribute among the total available binding sites. The extinction at the wave-length maximum (504 mμ) of bound, unstacked acridine orange therefore rises[1]. As can be seen in Fig. 2, the rate of unstacking is faster in deoxyribonucleic acids which have not been heated above the transition temperature. The fractional decrease in unstacking rate (expressed as $dE/d(P/D)$, where P/D is the number of deoxyribonucleic acid phosphates per acridine orange ion) accompanying denaturation appears to lead the changes in other properties by about 5° C. (Table 1), suggesting that deoxyribonucleic acid may undergo another structural transition which precedes that measured by viscosity, ultraviolet absorption, and β/α ratio changes.

As unstacking proceeds with increasing P/D ratio the α (monomer) band increases and β (dimer) band falls. The P/D at which the β/α ratio has fallen to unity may be used as a third measure of stacking tendency, and as shown (Table 1, Fig. 1) this measure also follows quantitatively the viscosity–ultra-violet absorption transition.

When the titration is carried to sufficiently high P/D (about 100 for deoxyribonucleates) the extinction at 504 mμ reaches a plateau corresponding to complete unstacking. It has been shown[1] that the fraction of unstacked acridine orange, F, is related to P/D by a single-parameter equation :

$$P/D = (1-F^{1/2})^{-1} + (K-1)(1-F^{1/2})^{-1}(1+F-F^{1/2})F^{1/2}$$

in which K, the stacking coefficient, is an absolute measure of stacking tendency defined in terms of a mathematical model of the stacking process. The value of K calculated from the plot of E_{504} vs. P/D turns out to be $1{\cdot}16 \pm 0{\cdot}14$ for calf thymus deoxyribonucleic acid and very close to this value for a number of other preparations (Stone, A. L., and Bradley, D. F., unpublished results). A value of $K = 1$ corresponds to no stacking tendency, that is, to a completely random distribution of dyes among sites, and therefore native deoxyribonucleic acid, with a K value close to unity, nearly corresponds to this situation. This finding is in agreement with Lawley's[7] conclusion that rosaniline dimerizes on native deoxyribonucleic acid in "tolerable agreement" with a random distribution model.

It has been reported[1] that deoxyribonucleic acid and ribonucleic acid have similar stacking coefficients (with $K = 2{\cdot}9$ and $3{\cdot}3$ respectively). It is apparent from the present findings that this conclusion was based upon experiments with partially denatured salmon sperm deoxyribonucleic acid (Table 1). Indeed, a comparison of the various measures of stacking suggests that unheated ribonucleic acid is more like denatured deoxyribonucleic acid than native deoxyribonucleic acid in those structural features responsible for dye stacking, for example, in having regions of both coiled and helix structure[8].

The data for the unstacking of boiled deoxyribonucleic acid, however, do not show good fit to the stacking equation ($K = 5{\cdot}36 \pm 2{\cdot}44$). A normal stacking curve (K about 3) is followed up to approximately 40 per cent unstacking. Beyond this, unstacking proceeds at a much slower rate, which is linear in log P/D, resulting in a higher average stacking coefficient than ribonucleic acid. This apparent increase in stacking tendency or flexibility at $P/D > 4$ may possibly be related to changes in the net charge on the deoxyribonucleic acid as the fraction of sites occupied by the acridine orange cations decreases, or to the presence in the boiled sample of a mixture of deoxyribonucleates with different binding affinities and stacking tendencies.

These results show that the tendency of acridine orange to aggregate is quite low when bound to native deoxyribonucleic acid, but that it increases markedly as the deoxyribonucleic acid is denatured. Several quantitative measures of stacking tendency have been described which may be used to estimate the extent of denaturation in partially denatured deoxyribonucleates. These findings provide additional evidence that the stacking tendencies of polyanions are intimately related to their structure in a qualitatively predictable manner. Furthermore, they may provide an important clue to understanding the complex colour patterns observed when acridine orange is used to stain tissues and, in particular, the general shift to dimeric character (enhancement of red phosphorescence) when living, stained cells are destroyed[3].

We wish to express our appreciation to Dr. A. L. Stone for helpful discussions, to Mrs. J. Johnson and Mrs. S. Huang for technical assistance, to Dr. S. Luborsky for a sample of soluble ribonucleic acid, and to Dr. J. S. Colter for a sample of mouse ascites tumour ribonucleic acid. One of us (G. F.) has been supported in part by a grant (*C*-3883) from the National Cancer Institute, U.S. Public Health Service.

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EFFECT OF CHLORPROMAZINE UPON ISOLATED *d*-AMINO-ACID OXIDASE SYSTEMS

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YAGI, Nagatsu and Ozawa[1] reported recently that chlorpromazine (N-[3′-dimethylaminopropyl]-3-chlorophenothiazine) is an inhibitor of *d*-amino-acid oxidase. They stated that substantial inhibition was achieved with 2×10^{-5} *M* chlorpromazine in a system consisting of a hog kidney *d*-amino-acid oxidase preparation, $1{\cdot}6 \times 10^{-7}$ *M* flavin adenine dinucleotide, and $6{\cdot}25 \times 10^{-2}$ *M* *d*-alanine, buffered at *p*H 8·3 with pyrophosphate (no temperature was given, nor the gas phase indicated). They also noted that marked inhibition was observed with the same concentration of chlorpromazine in the oxygen uptake of a freshly excised dog diencephalon homogenate, in which the endogenous respiration was inhibited by potassium cyanide; this phenomenon was attributed to the inhibition by chlorpromazine of *d*-amino-acid oxidase in the tissue, and it was suggested that there might be some relationship between the inhibition of flavin enzymes by chlorpromazine and the pharmacodynamic properties of the tranquillizing agent. In connexion with a study of the effect of gradual changes in the molecular constitution of synthetic entities upon isolated enzyme systems known to be associated with brain metabolism, we became interested in pursuing this work further.

Procedure 1. The work was carried out on a *GME*-Lardy *WB*-3 type Warburg instrument, using 15-ml. flasks. We have chosen Worthington's hog kidney *d*-amino-acid oxidase preparation and Calbiochem's flavin adenine dinucleotide (assay, 91 per cent, California Corporation for Biochemical Research, Los Angeles, California) for our initial experiments: 1×10^{-1} *M* *dl*-alanine was adopted as substrate after a study of the enzyme activity under varying enzyme and substrate concentrations (Worthington's

d-amino-acid oxidase preparation is prepared by extracting the conventionally prepared hog kidney acetone powder with distilled water for 1 hr. in the cold, centrifuging the mixture, and lyophilizing the extract immediately. Worthington Biochemical Corporation, Freehold, New Jersey). The buffers used were: 0·05 *M* KH_2PO_4–NaOH (*p*H 6·88), 0·025 *M* $Na_4P_2O_7$–HCl (*p*H 7·93), and 0·02 *M* $Na_4P_2O_7$–HCl (*p*H 8·27). The gas phase was air, and the temperature was 37° C. The reaction volume totalled 3·2 ml., with 0·12 ml. 20 per cent potassium hydroxide in the centre well (2 cm. × 2 cm. Whatman No. 1 filter paper), and 0·4 ml. substrate solution in the side-arm; all other reaction components were contained in the main compartment. The inhibitors were introduced in 0·05 ml. of aqueous solution, appropriately concentrated to yield the desired dilution of the compounds in the final reaction mixture. Equilibration was initiated at − 45 min., with readings every 5 min. beginning at − 25 min. Dumping was effected at 0 min., with the side-arm being washed twice with the reaction mixture. Each manometer was read at 10-min. intervals during the reaction period of 0 to + 60 min. The *p*H of the contents of the flasks was checked at the end of the experiment. Each set of conditions was run (at least) in triplicate.

Procedure 2. Some of the experiments required a change in the original arrangement (procedure 1) of the reaction components and the following modification was introduced: the enzyme and inhibitor solutions (combined volume 0·65 ml.) were placed in the side-arm, and the main compartment contained the coenzyme and substrate in the buffer balance (combined volume 2·43 ml.). Otherwise the same procedure was followed.

We expressed the rate as:

$$V = \left[\frac{(\mu\text{l. oxygen at 40 min.}) - (\mu\text{l. oxygen at 10 min.})}{30}\right] \times 60$$

where V signifies µl. oxygen/hr. absorbed within the reaction interval of + 10 to + 40 min., during which the rate was always linear.

Procedure 3. In experiments where the reaction was not preceded by an incubation period, the reactants were situated in accordance with the original arrangement (procedure 1); however, dumping was effected just prior to their exposure to the water-bath. In this instance, the rate (V) was calculated from the reaction interval of + 30 to + 50 min., during which the rate was always linear.

The percentage of inhibition (I) and potentiation (P) were calculated as follows:

$$I = \left(\frac{V_c - V_i}{V_c}\right) \times 100 \qquad P = \left(\frac{V_p - V_c}{V_c}\right) \times 100$$

where V_c represents the control rate, V_i the inhibited rate, and V_p the potentiated rate.

The chlorpromazine hydrochloride used in our experiments was furnished by Dr. Paul Mattis of the Smith, Kline and French Laboratories in Philadelphia, Pa., the reserpine phosphate by Dr. Albert J. Plummer, of Ciba Pharmaceuticals in Summit, N.J., *Ro*-1-9569/7 by Dr. W. E. Scott, of Hoffmann-LaRoche, Inc., in Nutley, N.J., and the *d*-lysergic acid diethylamide by Mr. John M. Cole, of Sandoz Pharmaceuticals in Hanover, N.J.

The enzyme systems used in the experiments were characterized kinetically (procedure 1). A linear reciprocal Lineweaver–Burk plot[3] of the enzyme activity, buffered with phosphate at *p*H 6·88, at varying enzyme and substrate concentrations, was graphically evaluated as suggested by Dixon[3] and Reiner[4]; the Briggs–Haldane constants[4a,5] (0·059, 0·044, 0·041 and 0·051) did not vary significantly with enzyme concentration and were well within the range of variability for independent determinations in the systems investigated (Reiner, J. M., personal communication, 1959). In pyrophosphate-buffered systems (*p*H 8·27), however, the enzyme activity did not yield a linear reciprocal plot; further discussion of this point is reserved for a future occasion.

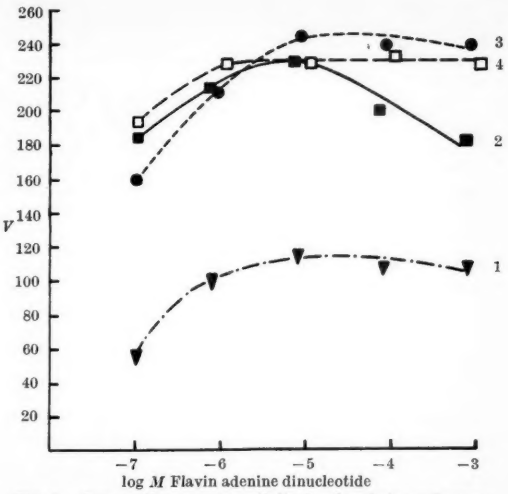

Fig. 1. Comparative enzyme activity in phosphate- and pyrophosphate-buffered systems (in curves 1, 2 and 3, coenzyme incubated with enzyme according to procedure 1; in curve 4, coenzyme incubated with substrate according to procedure 2). Common: 1×10^{-1} *M* *dl*-alanine, base level flavin adenine dinucleotide (estimated at 1×10^{-7} *M*/1 × *d*-amino-acid oxidase). Curve 1, 1 × *d*-amino-acid oxidase plus $7{\cdot}83 \times 10^{-7}$ to $7{\cdot}83 \times 10^{-4}$ *M* flavin adenine dinucleotide, 0·05 *M* phosphate, *p*H 6·88. Curve 2, 1 × *d*-amino-acid oxidase plus $6{\cdot}73 \times 10^{-7}$ to $6{\cdot}73 \times 10^{-4}$ *M* flavin adenine dinucleotide, 0·02 *M* pyrophosphate, *p*H 8·27. Curve 3, 2·33 × *d*-amino-acid oxidase plus $9{\cdot}19 \times 10^{-7}$ to $9{\cdot}19 \times 10^{-4}$ *M* flavin adenine dinucleotide, 0·05 *M* phosphate, *p*H 6·88. Curve 4, 1 × *d*-amino-acid oxidase plus $1{\cdot}05 \times 10^{-6}$ to $1{\cdot}05 \times 10^{-3}$ *M* flavin adenine dinucleotide, 0·02 *M* pyrophosphate, *p*H 8·27

Another kinetic study revealed additional differences between enzyme activities in phosphate (*p*H 6·88) and pyrophosphate (*p*H 8·27) buffered systems (Fig. 1). Curves 1, 2 and 3 are results obtained with procedure 1 in which the enzyme and the coenzyme were pre-incubated in the same compartment. Curves 1 and 2 represent the activity of a given quantity of *d*-amino-acid oxidase and tenth-molar *dl*-alanine within a range of concentrations of flavin adenine dinucleotide, in phosphate- and pyrophosphate-buffered systems. Curve 3 represents the corresponding activity of 2·33 times the amount of *d*-amino-acid oxidase used in the first two series of experiments, which raised the oxygen uptake in the phosphate buffer to approximately the same level as that of the original enzyme concentration in the pyrophosphate buffer. While the oxygen uptake differed quantitatively in the two phosphate-buffered experiments (Fig. 1, curves 1 and 3), corresponding to the enzyme concentration, the curves of the flavin adenine dinucleotide catalysis retained the same form. In the pyrophosphate-buffered experiment, however, the graph of the activity took on a considerably different pattern (Fig. 1, curve 2).

Table 1. Effect of Pre-incubation upon the Activity of the Hog Kidney *d*-Amino-acid Oxidase Preparation
(1 × *d*-amino-acid-oxidase with base-level flavin adenine dinucleotide (estimated, 1×10^{-7} *M*) and 1×10^{-1} *M dl*-alanine)

Buffer (*M*) and *p*H	Pre-incubation period at 37° C. prior to reaction (min.)	
	45	0
Pyrophosphate (0·025) 8·04	$V = 183$	$V = 180$
Phosphate (0·05) 6·84	$V = 76$	$V = 78$

When the coenzyme and the enzyme were pre-incubated in separate compartments (procedure 2), the pyrophosphate-buffered run yielded curve 4 of Fig. 1; the phosphate-buffered runs carried out under these conditions did not differ significantly from those represented by curves 1 and 3.

Since Burton has found[6] that some *d*-amino-acid oxidase preparations are subject to thermal inactivation at 37·5° C. in pyrophosphate-buffered (*p*H 8·27) systems, we have compared the activity of our enzyme systems in simultaneous runs with (procedure 1) and without (procedure 3) a 45 min. pre-incubation period; the results are summarized in Table 1. While our enzyme systems were not affected by pre-incubation at 37° C., one should bear in mind that Burton apparently dealt with a purer and more sensitive sheep kidney preparation.

We observed a 40 per cent inhibition with 1×10^{-3} *M* chlorpromazine in phosphate buffer (*p*H 6·88, procedure 1), but were unable to effect appreciable chlorpromazine inhibition in pyrophosphate buffers (*p*H 7·93 and 8·27) under similar conditions. The inhibition in phosphate-buffered systems was substantially influenced by the flavin adenine dinucleotide concentration of the reaction mixture and by the exposure of the enzyme to the inhibitor during pre-incubation. Our findings are summarized in Table 2.

The effect of chlorpromazine upon phosphate-buffered systems with increasing flavin adenine dinucleotide concentrations is illustrated in Fig. 2 (curve 2). The phenomenon of chlorpromazine potentiation at high concentrations of flavin adenine dinucleotide seems to be correlated with some inhibition by flavin adenine dinucleotide itself at such coenzyme-levels (Fig. 2, curve 1), which tendency becomes even more conspicuous in curve 2 of Fig. 1. This prompts the suggestion that complex formation between chlorpromazine and flavin adenine dinucleotide may occur. Reiner[4,5] actually worked out a mathematical treatment for cases in which an inhibitor effects potentiation by interacting with a second inhibitory substance present in the reaction mixture. Complex formation between flavin adenine dinucleotide and chlorpromazine is chemically also conceivable by means of charge effects between the pyrophosphate moiety of the flavin adenine dinucleotide and a basic function (or functions) of the chlorpromazine molecule within some *p*H ranges.

In order to compare the effect of flavin adenine dinucleotide upon the inhibition of *d*-amino-acid oxidase by chlorpromazine prior and subsequent to pre-incubation, we ran a series of experiments summarized in Table 3. It is interesting that, when

Table 2. Comparative Effects of Chlorpromazine and Nicotinic Acid upon Isolated *d*-Amino-acid Oxidase Systems
(1 × *d*-amino-acid oxidase and 1×10^{-1} *M dl*-alanine)

Buffer (*M*) and *p*H	Pre-incubation period at 37° C. prior to reaction in minutes													
	Chlorpromazine (1×10^{-3} *M*)								Nicotinic acid (1×10^{-3} *M*)					
	45				0				45			0		
	FAD (*M*)	V_e	*I*%	*P*%	FAD (*M*)	V_e	*I*%	*P*%	FAD (*M*)	V_e	*I*%	FAD (*M*)	V_e	*I*%
Pyrophosphate (0·025) 7·92–7·93	B.L.* + $7{\cdot}70 \times 10^{-6}$	202	—	8	B.L. + $1{\cdot}00 \times 10^{-5}$	199	—	18	B.L. + $1{\cdot}38 \times 10^{-5}$	200	33	B.L. + $1{\cdot}00 \times 10^{-5}$	199	30
,,	B.L.	164	5	—	B.L.	179	—	3	B.L.	162	31	B.L.	179	29
Phosphate (0·05) 6·84–6·88	B.L.	60	40	—	B.L.	70	—	4	B.L.	60	37	B.L.	70	39
,,	B.L. + $7{\cdot}58 \times 10^{-6}$	118†	—	5	B.L. + $2{\cdot}00 \times 10^{-5}$	130	—	27	B.L. + $8{\cdot}04 \times 10^{-6}$	118†	52	B.L. + $2{\cdot}00 \times 10^{-5}$	130	42

* B.L. = base level (estimated: 1×10^{-7} *M*).
† $7{\cdot}29 \times 10^{-6}$ *M* flavin adenine dinucleotide in V_e.

Table 3. Effect of Flavin Adenine Dinucleotide upon Chlorpromazine Inhibition of *d*-Amino-acid Oxidase Prior and Subsequent to Pre-incubation
(1 × *d*-amino-acid oxidase and 1×10^{-1} *M dl*-alanine)

Buffer (*M*) and *p*H	Arrangement of reactants during the 45 min. pre-incubation period at 37° C.						
	Main comp.: enzyme, coenzyme and inhibitor; comb. vol. 2·68 ml. Side-arm: Substrate; comb. vol. 0·40 ml.				Main comp.: coenzyme and substrate; comb. vol. 2·43 ml. Side-arm: enzyme and inhibitor; comb. vol. 0·65 ml.		
	FAD (*M*)	V_e	*I* per cent	*P* per cent	FAD (*M*)	V_e	*I* per cent
Pyrophosphate (0·025) 7·93–7·99	B.L.* + $7{\cdot}70 \times 10^{-6}$	202	—	8	B.L. + $6{\cdot}54 \times 10^{-4}$	201	23
,,	B.L.	164	5	—	B.L.	175	25
Phosphate (0·05) 6·84–6·88	B.L.	60	40	—	B.L.	65	62
,,	B.L. + $7{\cdot}58 \times 10^{-6}$	118†	—	5	B.L. + $6{\cdot}54 \times 10^{-4}$	107	65

* B.L. = base level (estimated: 1×10^{-7} *M*).
† $7{\cdot}29 \times 10^{-6}$ *M* flavin adenine dinucleotide in V_e.

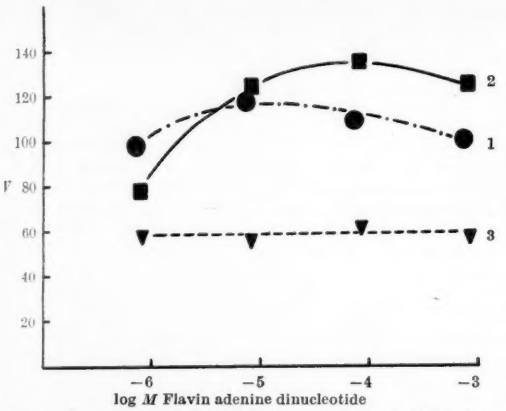

Fig. 2. Comparative effects of chlorpromazine and nicotinic acid in phosphate-buffered systems (procedure 1). Common: 1×10^{-1} *M dl*-alanine, base-level flavin adenine dinucleotide (estimated at 1×10^{-7} *M*), 1 × *d*-amino-acid oxidase, 0·05 *M* phosphate, *p*H 6·88. Curve 1, plus $7{\cdot}27 \times 10^{-7}$ to $7{\cdot}27 \times 10^{-4}$ *M* flavin adenine dinucleotide. Curve 2, plus $7{\cdot}58 \times 10^{-7}$ to $7{\cdot}58 \times 10^{-4}$ *M* flavin adenine dinucleotide and 1×10^{-3} *M* chlorpromazine. Curve 3, plus $8{\cdot}04 \times 10^{-7}$ to $8{\cdot}04 \times 10^{-4}$ *M* flavin adenine dinucleotide and 1×10^{-3} *M* nicotinic acid

excess flavin adenine dinucleotide was combined with the enzyme-inhibitor mixture subsequent to pre-incubation (procedure 2), the coenzyme would not reverse or even decrease the inhibition; yet the flavin adenine dinucleotide content of the final reaction mixture was about a hundred times that which effectively counteracted inhibition when the coenzyme was pre-incubated with the enzyme-inhibitor mixture (procedure 1). (The small volume for the pre-incubation of the enzyme-inhibitor mixture in procedure 2 was chosen to effect significant inhibition in the pyrophosphate-buffered experiments.)

The information at hand indicates that formation of the enzyme-chlorpromazine complex proceeds through a relatively slow reaction, and that once the complex has formed, it is not easily reversible. One could visualize a limited structural analogy between the N-alkyl substituted phenothiazine moiety of chlorpromazine and the N-ribityl substituted *iso*alloxazine component of flavin adenine dinucleotide, which may account for both (1) the stability of the chlorpromazine-enzyme complex and (2) the preferential affinity of the *free* apoenzyme for flavin adenine dinucleotide.

In comparative experiments with nicotinic acid, which is known to effect inhibition by reversible competition with *d*-alanine for the combined apo- and co-enzyme[7], the chlorpromazine inhibition was approximately of the same magnitude as that of the reference reagent (procedure 1, phosphate buffer, *p*H 6·88). The nicotinic acid inhibition, however, was not affected significantly by the type of buffer, concentration of flavin adenine dinucleotide or pre-incubation (see Table 2, and Fig. 2).

In conclusion, it should be noted that reserpine, Hoffmann-LaRoche's *Ro*-1-9569/7, *d*-lysergic acid diethylamide and cocaine had no significant effects upon *d*-amino-acid oxidase systems (procedure 1) optimal for chlorpromazine inhibition.

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EQUILIBRIUM CONSTANT OF THE GALACTOKINASE REACTION AND FREE ENERGY OF HYDROLYSIS OF ADENOSINE TRIPHOSPHATE

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THE adenosine triphosphate–adenosine diphosphate system appears to be a universal component of living cells, and is required for activation and utilization of most metabolites. A reliable estimate of the free-energy change for hydrolysis of adenosine triphosphate is therefore needed for evaluation of the energetics of the processes concerned with cell growth and maintenance. Early estimates, based on thermal data and some equilibrium constants, indicated that the standard free-energy change ($\Delta G'$) for the reaction:

$$\text{adenosine triphosphate} + \text{water} \rightarrow \text{adenosine diphosphate} + \text{orthophosphate} \quad (1)$$

was between −9 and −12 kcal./mole at *p*H 7 (ref. 1). More recently, the free-energy change for this reaction has been calculated from the equilibrium constants of a number of phosphoryl transfer reactions and less negative values (−5 to −8 kcal./mole at *p*H 7) have been reported[2–4]. The discrepancies between published values are due chiefly to the difficulty of accurately determining the equilibrium concentrations of the minor components of reactions for which the change of free energy is large.

The free-energy change of reaction (1) may be obtained as the sum of the free energies of phosphoryl transfers from adenosine triphosphate to hexoses, and from hexose phosphates to water. Previous workers[2,3] have used the reactions catalysed by hexokinase, that is:

$$\text{adenosine triphosphate} + (\alpha+\beta)\text{-D-glucose} \rightarrow \text{adenosine diphosphate} + (\alpha+\beta)\text{-D-glucose-6-phosphate} \quad (2)$$

and by phosphatase, that is:

$$(\alpha+\beta)\text{-D-glucose-6-phosphate} + \text{water} \rightarrow (\alpha+\beta)\text{-D-glucose} + \text{orthophosphate} \quad (3)$$

However, these reactions are inconvenient because of the large free-energy change ($-2{\cdot}9$ [2] or $-4{\cdot}4$ [3] kcal./mole at *p*H 7) of reaction (2). For the reaction catalysed by phosphoglucomutase, that is:

α-D-glucose-1-phosphate → (α+β)-D-glucose-6-phosphate (4)

the free-energy change is $-1{\cdot}7$ kcal./mole at *p*H 7 and 25° C.[5]. It is evident that the equilibrium constant of phosphoryl transfer from adenosine triphosphate to carbon 1 of hexose may be more accurately measured than that of phosphoryl transfer to carbon 6.

In the present work, the equilibrium constant of the reaction catalysed by galactokinase, that is:

adenosine triphosphate + (α+β)-D-galactose → adenosine diphosphate + α-D-galactose-1-phosphate (5)

has been measured.

The reaction was carried out at *p*H 7·0 and 25° C. with 25 m*M* magnesium chloride, which gave an excess of Mg^{2+} at the concentrations of nucleotides used. The initial reactants were adenosine triphosphate and galactose, or adenosine diphosphate and α-D-galactose-1-phosphate. The course of the reaction was followed by measurement of acid or alkali uptake at *p*H 7·0 after addition of yeast galactokinase and muscle adenylate kinase. The latter enzyme catalyses the reaction:

2 adenosine diphosphate → adenosine triphosphate + adenosine monophosphate (6)

and facilitates accurate measurement, by keeping the ratio adenosine triphosphate/adenosine diphosphate close to unity. Nucleotides were estimated by specific enzymic and electrophoretic methods, hexose phosphates were measured enzymically and hexoses by enzymic and colorimetric methods[6].

As shown in Table 1, the equilibrium constant for reaction (5) in the forward direction was 25 ± 3, and in the reverse direction was 27 ± 3. In a preliminary series of six experiments (with 10 m*M* magnesium chloride), the equilibrium constant was 25 ± 9. The standard free-energy change of reaction (5) is therefore $-1{\cdot}9 \pm 0{\cdot}1$ kcal./mole at *p*H 7·0 and 25° C. in the presence of an excess of magnesium ions.

The free-energy change of the reaction:

α-D-galactose-1-phosphate → α-D-glucose-1-phosphate (7)

is $-0{\cdot}7$ kcal./mole[7]. This reaction involves conversion of the axial hydroxyl group at carbon 4 to an equatorial configuration, and the free-energy does not differ significantly from that of the corresponding transformation in other compounds[8,9]. Hence the free energy of hydrolysis of α-D-galactose-1-phosphate and α-D-glucose-1-phosphate must be independent of the orientation of the remote hydroxyl at carbon 4. The free energy of hydrolysis of α-D-galactose-1-phosphate has therefore been estimated from the free-energy changes of reactions (3) and (4).

The equilibrium constant of reaction (3) was determined in both directions at *p*H 7·0 and 25° C. in the presence of Mg^{2+} and purified intestinal alkaline phosphatase[10]. As shown in Table 2, the free-energy change of reaction (3) is $-3{\cdot}3 \pm 0{\cdot}1$ kcal./mole in these conditions. This is in agreement with the value obtained by Meyerhof and Green[11] with more concentrated reactants, but is 0·7 kcal./mole more negative than the value reported by Guinodman[12].

The equilibrium constant of reaction (4) was also measured in both directions at *p*H 7·0 and 25° C. in the presence of Mg^{2+} and purified muscle phosphoglucomutase[10]. As shown in Table 3, the free-energy change of this reaction was $-1{\cdot}7 \pm 0{\cdot}1$ kcal./mole. Colowick and Sutherland[5] also obtained a value of $-1{\cdot}7$ kcal./mole for this reaction.

For the hydrolysis of α-D-galactose-1-phosphate, that is:

α-D-galactose-1-phosphate + water → (α+β)-D-galactose + orthophosphate (8)

the free-energy change at *p*H 7·0 and 25° C. in the presence of an excess of Mg^{2+} is therefore $-5{\cdot}0$ kcal./mole.

From this value, and the free-energy change of reaction (5), the free energy of hydrolysis of adenosine

Table 1. Equilibrium Constant of Reaction (5) catalysed by Galactokinase (ref. 6)

Initial reactants	Concentration (m*M*) of products at *p*H 7·0, 25° C. and 25 m*M* Mg^{2+}*				*K*†	$\Delta G'^{\ddagger}$ (kcal./mole)
	Galactose	Galactose-1-phosphate	ATP	ADP		
ATP and galactose	$1{\cdot}2 \pm 0{\cdot}1$ (8)	$11{\cdot}6 \pm 0{\cdot}1$ (8)	$1{\cdot}06 \pm 0{\cdot}02$ (4)	$2{\cdot}78 \pm 0{\cdot}03$ (4)	25 ± 3	$-1{\cdot}9 \pm 0{\cdot}1$
ADP and α-D-galactose-1-phosphate	$0{\cdot}37 \pm 0{\cdot}04$ (8)	$6{\cdot}1 \pm 0{\cdot}1$ (8)	$1{\cdot}51 \pm 0{\cdot}04$ (4)	$2{\cdot}49 \pm 0{\cdot}01$ (4)	27 ± 3	$-1{\cdot}9 \pm 0{\cdot}1$

* Results given as mean ± *S.E.*, with the number of determinations in parentheses.

† $K = \dfrac{[\text{ADP}]\,[\alpha\text{-D-galactose-1-phosphate}]}{[\text{ATP}]\,[(\alpha+\beta)\text{-D-galactose}]}$

‡ $\Delta G' = -RT\ln K$.

Table 2. Equilibrium Constant of Reaction (3) catalysed by Phosphatase (ref. 10)

Initial reactants	Concentrations (m*M*) of products at *p*H 7·0, 25° C.				*K**	$\Delta G'$ (kcal./mole)
	Orthophosphate	Glucose	Glucose-6-phosphate	Mg^{2+}		
Glucose and orthophosphate	163	151	0·074	0	332	−3·5
	248	214	0·200	2	266	−3·3
	246	239	0·225	5	261	−3·3
Glucose and orthophosphate and glucose-6-phosphate (approx. 1 m*M*)	240	248	0·33	2	181	−3·1
	167	253	0·156	2	271	−3·3

* $K = \dfrac{[(\alpha+\beta)\text{-D-glucose}]\,[\text{orthophosphate}]}{[(\alpha+\beta)\text{-D-glucose-6-phosphate}]}$

Table 3. EQUILIBRIUM CONSTANT OF REACTION (4) CATALYSED BY PHOSPHOGLUCOMUTASE (ref. 10)

Initial reactants	Concentrations (mM) of products at pH 7·0, 25° C. and 25 mM Mg^{2+}		K^*	$\Delta G'$ (kcal./mole)
	(α+β)-D-glucose-6-phosphate	α-D-glucose-1-phosphate		
Glucose-6-phosphate	13·6	0·77	18	−1·7
Glucose-1-phosphate	12·6	0·90	14	−1·6
Glucose-6-phosphate and glucose-1-phosphate	2·09	0·11	19	−1·7

$$^*K = \frac{[(\alpha+\beta)\text{-D-glucose-6-phosphate}]}{[\alpha\text{-D-glucose-1-phosphate}]}$$

triphosphate at pH 7·0 and 25° C. in the presence of an excess of Mg^{2+} is −6·9 kcal./mole. The standard error for this value is probably less than 0·2 kcal./mole.

Benzinger, Kitzinger, Hems and Burton[4] have recently determined the free-energy change for the hydrolysis of glutamine, that is:

$$\text{glutamine} + \text{water} \rightarrow \text{glutamate} + \text{ammonia} \quad (9)$$

Combination of their value with estimates by other workers[13,14] of the free-energy change of the reaction:

$$\text{adenosine triphosphate} + \text{ammonia} + \text{glutamate} \rightarrow \text{adenosine diphosphate} + \text{glutamine} \quad (10)$$

indicates that the free-energy change of reaction (1) is −6·8 kcal./mole at pH 7·0 and 25° C. in the presence of an excess of Mg^{2+}.

By combination of the free-energy change of reaction (3) as reported here (−3·3 kcal./mole) and the free-energy change of reaction (2) as estimated by Robbins and Boyer[3], and by Vladimirov, Vlassova, Kolotilova, Lyzlova and Panteleyeva[2], the estimates of the free-energy change of reaction (1) at pH 7·0 and 25° C. in the presence of an excess of Mg^{2+} are −7·7 kcal./mole and −6·2 kcal./mole, respectively.

Biological phosphoryl transfers take place in the presence of Mg^{2+} and hence the free-energy values reported here are more relevant for energetics of physiological processes than the much more negative values obtained in the absence of Mg^{2+}.

The free energies of phosphoryl transfers from adenosine triphosphate to many other metabolites are known. By combining these values with the value of the free energy of hydrolysis of adenosine triphosphate established here, reliable estimates of the free energies of hydrolysis of most phosphorylated metabolites have now been obtained[15,16].

A full account of the work described here will be submitted for publication elsewhere[6,10,16]. We wish to thank Dr. R. M. Burton, Washington University, St. Louis, for generously supplying the purified galactokinase used in these studies.

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COMPLEMENTATION AMONG THE SUB-GENIC MUTANTS IN THE *r*-LOCUS OF *DROSOPHILA MELANOGASTER*

By Dr. O. G. FAHMY and Dr. MYRTLE J. FAHMY

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, S.W.3

THE composite nature of the gene, both as regards structure and function, has been admirably demonstrated by Benzer's[1] work on the *r*-II locus of the T_4 bacteriophage. That complex genes are of widespread (if not universal) occurrence has also been demonstrated by the discovery of pseudoallelic series in a great diversity of organisms: *Aspergillus*[2], *Neurospora*[3], and *Drosophila*[2,4]. The description of the various attributes of a gene naturally necessitated a new terminology, which has now been adequately formulated by Benzer[1]. He differentiated between the unit of recombination: the 'recon', the unit of mutation: the 'muton', and the unit of function: the 'cistron'. Two mutants within a locus are assigned to the same cistron if they express the mutant phenotype in the *trans* position, that is, when occurring in repulsion on the homologous chromosomes. The corollary to this situation is the *cis* arrangement: when the two mutants are in coupling on a member of a pair of homologues, with the non-mutant allelomorphs on the other, and this exhibits the 'normal' or wild-type phenotype. Applied to *Drosophila*, this terminology means that a pair of pseudoallelic mutants occur in the same cistron if they give an interaction phenotype (that is, exhibit the mutation) in the F_1 heterozygote. Conversely, the interallelic mutants are in different cistrons if they give complementation in the F_1, expressing the wild-type phenotype. Inter-allelic complementation has been discovered in *Drosophila* at the *rudimentary* locus, and this phenomenon will be analysed in this communication.

Induction of the Mutants

In the course of our work[5] on the induction of sex-linked recessive visibles by radiation, as well as a

Table 1. Distribution of the Mutant Types and the Affected Cistrons under Various Mutagens

Cistrons Mutant types	I *a*	I + II *e*	II + III *g*	III *b*	IV + V *f*	V *c*	VI *d*	I — IV *h*	I — VI *i*	Total
Mutagens										
Amino-acid mustards (*CB.* 1592, 3007, 3025, 3026)	1	1	1	1	—	1	1	—	3	9
Alkyl methanesulphonates (*CB.* 1506, 1522, 1528, 1540, 2041)	—	—	—	—	1	4	1	1	7	14
Phenylethylamine mustard (*CB.* 3034)	—	—	—	—	—	1	—	—	2	3
X-rays	—	—	—	—	—	—	2	—	2	4
Stock *r* (spontaneous)	—	—	—	—	—	1	—	—	—	1
Total	4				27					31

diversity of alkylating mutagens (chloroethylamines, polyethyleneimines, and methanesulphonic esters), a total of 68 mutants at the *rudimentary* (*r*) locus were recovered. Of these, 31 mutants were retained for the inter-allelic tests. A few of the mutants analysed were induced by X-radiation; the remainder were induced by various alkylating compounds (Table 1). These compounds, together with their Chester Beatty (*CB*) code numbers and chemical names, will be listed here under their appropriate chemical series.

Amino-acid mustards: CB 1592, S-2-chloroethylcysteine; *CB* 3007, 3025 and 3026, the DL-, the L-, and D- isomers of *p*-NN-di-(2-chloroethyl)aminophenylalanine.

Phenylethylamine mustard: CB 3034, *p*-NN-di-(2-chloroethyl)aminophenylethylamine.

Alkyl methanesulphonates: CB 1506, 2-chloroethyl methanesulphonate; *CB* 1522, 2-fluorethyl methanesulphonate; *CB* 1528 ethyl methanesulphonate; *CB* 1540, methyl methanesulphonate; *CB* 2041, 1 : 4-dimethanesulphonoxybutane.

Phenotypically, all the *r* mutants were fairly comparable, and in general they resembled the stock visible which has already been described in the literature[6]. The hemizygous *r* males, as well as the homozygous females, were characterized by short obliquely truncated wings, with sparse costal bristles. When allelism tests were undertaken between the various mutants, however, it became apparent that we were dealing with a pseudo-allelic series, probably involving several functional sub-units (cistrons). Some of the mutants gave the *r* phenotype in the heterozygote, while others resulted in full complementation, giving the wild type.

The Complementation Map

Extensive analysis was undertaken of the phenotypic expression of crosses involving 31 *r*-alleles, and this enabled the construction of the complementation map illustrated in Fig. 1. It was possible to arrange the mutants in a unique linear sequence involving different or adjacent cistrons. In the construction of this map, mutants which complemented were assigned

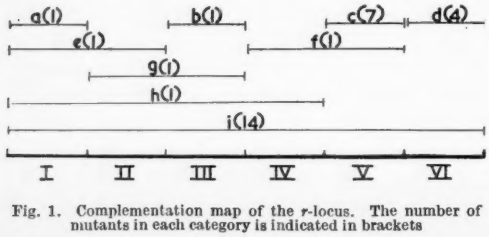

Fig. 1. Complementation map of the *r*-locus. The number of mutants in each category is indicated in brackets

to different functional units (cistrons). Thus mutants *a*, *b*, *c* and *d* (Fig. 1) complement in pairs and, therefore, belong to different cistrons. This fact by itself, however, does not permit any determination of the sequence of the affected sub-genes. The detection of the intra-locus linear organization is made possible through the presence of mutants resulting from multiple 'inactivations' (or damage), extending over more than one cistron. For example, mutant *e* interacts with *a*, but complements *b*; while *h* interacts with *a* and *b*, but complements *c*. This fixes the position of *b* between *a* and *c*.

The whole of the complementation map suggests the presence of at least 6-cistrons in the *r*-locus (Fig. 1). The majority of the mutants seem to involve more than one functional unit. Nearly half (14 out of the tested 31) are of the *i* type, involving all the 6-cistrons, and giving non-complementation to all other mutational types. These were at first thought to be small deficiencies at the *r*-locus. Careful cytological analysis of a non-complementing mutant (of the *i* type), however, failed to reveal even a single band deficiency in the salivary X-chromosome. According to the accepted criteria in *Drosophila* cytogenetics, therefore, even the multiple-cistron *r*-mutants must be looked upon as intra-genic point mutations. It is of interest to note that in *Neurospora*, as in *Drosophila*, non-complementing inter-allelic mutants were frequent, and did not prove to be the consequence of deletions[7]. Some of these mutants at the *ad*-4 locus of *Neurospora* underwent reversion by back-mutation, and afterwards yielded secondary forward mutants of the complementing types.

A phenotypic manifestation which has some bearing on the complementation map is the appearance of the costal wing-setæ, or marginal bristles. In some inter-mutant heterozygotes the phenotype is wild-type, except for the derangement and sparsity of the marginal wing bristles. This suggested a situation of 'partial' complementation, which was designated (r^+b), indicating an intermediate state between interaction giving the (r) phenotype and full complementation giving the (r^+), or wild-type expression. If this is so, it would be expected that the (r^+b) effect should have some bearing on the complementation relations between mutants, giving some indication of the proximity of the complementing types. Table 2 gives the F_1 phenotypic expression of crosses between complementing mutants in relation to the inter-mutant distance in terms of functional units (cistrons). It is convincingly clear that the proportion of (r^+b) decreases with the distance between the mutants, and completely disappears for alleles 3 or more cistrons apart. It is therefore certain that the (r^+b) phenotype is an indicator of proximity among the complementing mutants. In

Table 2. THE EXPRESSION OF THE 'BRISTLE-EFFECT' (r^+b) IN 92 INTER-ALLELIC CROSSES BETWEEN COMPLEMENTING *r*-MUTANTS IN RELATION TO INTER-MUTANT DISTANCE IN CISTRONS

Distance (in cistrons)	(r^+)	(r^+b)	Total	Proportion (r^+b)
0	23	19	42	0·45
1	13	6	19	0·32
2	12	4	16	0·25
3	11	0	11	0·00
4	4	0	4	0·00

fact it is on the basis of this criterion that mutant type *d* has been placed nearest to *c* (Fig. 1). This is because crosses between *c*- and *d*-mutants give the (r^+b) phenotype with the frequency expected for mutations affecting nearby cistrons. On the other hand, crosses of mutants $d \times a$ and $d \times e$ all gave full complementation.

The sample of *r*-mutants tested is much too small to permit an analysis of the distribution of the affected cistrons under individual mutagens. Nevertheless, when mutations by groups of related compounds are considered, certain suggestive tendencies become apparent (Table 1). The mutants under the amino-acid mustards seem to form a class of their own, appreciably distinct from the rest of the sample induced by the other agents. The small mutants (involving 1 or 2 cistrons) in the amino-acid mustard class show a larger scatter among the cistrons of the locus. In particular, 4 out of the 9 mutants in this class involved cistrons I to III, while none of the 22 mutants by all the other agents involved these cistrons individually, or in pairs. On the contrary, small mutations by agents other than the amino-acid mustards seem to occur exclusively in cistrons IV–VI. Even with the small samples of mutants available, a comparison of the above relative frequencies (that is, 4/9 compared to 0/22) did indicate a difference of decisive statistical significance ($P < 0{\cdot}01$). The lack of small mutants in cistrons I–III in the sulphonate sample as compared to that of the amino-acid mustards (that is, 0/14 compared to 4/9) also indicated a suggestively high difference ($P < 0{\cdot}05$). There is, therefore, some indication of a difference in the mode of action of the amino-acid mustards and the sulphonates on the sub-genic level, which recalls that which has been demonstrated on the genic level[5]. Experiments are now in progress to test the reality, and the degree, of the differential action on the *r*-cistrons under the effect of an individual compound belonging to each of the mutagenically contrasting chemical series : the amino-acid mustards and the sulphonates.

Mechanism and Significance of Allelic Complementation

Intra-genic complementation is a fairly widespread phenomenon in micro-organisms. The best-known examples occur at the *ad*-4[7,8] and *arg*-2[9] loci of *Neurospora*, but instances have also been reported in *Aspergillus*[10], as well as in *Salmonella*[11]. The physiological mechanism of complementation has now been adequately analysed[7,8] for the *ad*-4 locus of *Neurospora*. Extensive quantitative measurements of adenylosuccinase activity in complementing heterocaryons indicated that this activity ranges from 1 to 25 per cent that found in the parental strain. Further, a correlation existed between enzymatic activity and the distance between the mutants on the complementation map. Activity was maximal (that is, 25 per cent) for mutants separated by 4–6 cistrons, and fell gradually for nearer mutants, reaching a minimum when the damage occurred at juxtaposed cistrons. This biochemical situation in *Neurospora* is parallel to the phenotypic 'bristle-effect' occurring among some of the complementing *r*-mutants in *Drosophila*, and suggests an analogous physiological mechanism. It would seem likely that the (r^+b) phenotype is the outcome of a weak production of the r^+ 'determinant' (presumably an enzymatic complex), and that the amount of this 'determinant' increases with the distance between the damaged sub-loci in the heterozygote. The disappearance of the 'bristle-effect' when the damaged sub-units are 3 or more cistrons apart indicates that this is the minimal distance (in physiological units) which is needed before enough 'r^+ determinant' is produced to manifest the wild-type expression. It would be fascinating to determine the minimal distance for full phenotypic complementation in terms of physical entities (nucleotides) as well as in genetical (cross-over) units. So far, this has not been achieved either in *Neurospora* or in *Drosophila*. That this is within the scope of genetical techniques is indicated by some recent results[12] on the *pan*-2 locus of *Neurospora*, which show a correlation between order on the complementation map and the genetic distance in cross-over units. The analysis of the genetic map of the *r*-locus in *Drosophila* is now under investigation.

The complementation map here described for the *r*-locus of *Drosophila* is the first instance of this phenomenon discovered in higher organisms. Evidence of intra-locus complementation, however, has already been detected in our laboratory at two other *X*-chromosome loci : *tan* (*t*) and *minutechæte* (*mch*), and doubtless it must occur in many others. The occurrence of inter-allelic complementation in organisms ranging from bacteria to *Drosophila* emphasizes that it is a fundamental characteristic of gene structure and function. It implies that the linear sequence of the hereditary units is not only applicable to the arrangement of genes on chromosomes, but also is probably equally true of the ultimate organization of the genic substructure in terms of cistrons and nucleotides.

We wish to thank Mr. J. Maynard-Smith (University College, London) for his interest in this problem, and also for his helpful discussion.

This investigation has been supported by grants to the Chester Beatty Research Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

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FORTHCOMING EVENTS

*(Meeting marked with an asterisk * is open to the public)*

Tuesday, December 29

RADAR AND ELECTRONICS ASSOCIATION (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7.30 p.m.—Dr. K. R. Sturley: "High Quality Sound Broadcasting".*

Wednesday, December 30

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Dr. J. D. Carthy: "Animal Senses and Reactions". (First of two Juvenile Lectures. Further lecture on January 6.)

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 3 p.m.—Mr. G. G. Gouriet: "Colour Television". (Christmas Holiday Lecture. To be repeated on Thursday, December 31.)

Friday, January 1

PLASTICS INSTITUTE (in the Lecture Theatre of the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 2.30 p.m.—Dr. N. A. de Bruyne: "Sticky Plastics". (Sixth Lecture for Young People.)

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (preferably with an interest in the theory of machines, mechanical servomechanisms or the strength of materials) IN MECHANICAL ENGINEERING—The Secretary, The Queen's University, Belfast (January 1).

SENIOR LECTURER IN CLINICAL BIOCHEMISTRY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (January 4).

SENIOR LECTURER (with an honours degree and, preferably, a higher degree in electrical engineering, with specialization in electronics) IN ELECTRICAL ENGINEERING—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1, or The Registrar, University of Queensland, Brisbane, Queensland, Australia (Australia and London, January 15).

LECTURER IN BIOCHEMISTRY IN THE DEPARTMENT OF SURGERY to work on problems related to the formation of pre-collagen and collagen in wound healing—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (January 16).

LECTURER/SENIOR LECTURER IN NUTRITION to undertake teaching and research in livestock nutrition in the Department of Nutrition and Chemical Pathology, Faculty of Rural Science, University of New England, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, January 20).

ASSISTANT LECTURER IN STATISTICS, to teach theoretical statistics and some mathematics—The Secretary, The London School of Economics and Political Science, Houghton Street, Aldwych, London, W.C.2 (January 22).

BABCOCK AND WILCOX FELLOW IN NUCLEAR ENGINEERING—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (January 30).

COAL PETROLOGIST (graduate majoring in geology, or with equivalent qualifications, and considerable experience in the field of coal petrology) IN THE COAL RESEARCH SECTION, Commonwealth Scientific and Industrial Research Organization, Sydney, New South Wales, Australia, to carry out investigations on the microscopy of coals and cokes—The Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 480/324 (January 30).

SENIOR TUTOR-DEMONSTRATOR IN PHYSICS at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, January 30).

SENIOR LECTURER/LECTURER IN CHEMICAL AND EXTRACTION METALLURGY at the University of New South Wales, Australia—The Agent General for New South Wales, 56–57 Strand, London, W.C.2 (Australia, January 31).

LECTURER or ASSISTANT LECTURER (with a special interest in climatology and/or biogeography and prepared to take part in the teaching of practical cartography) IN GEOGRAPHY—The Secretary and Registrar, The University, Southampton (February 1).

LECTURER or ASSISTANT LECTURER (with a special interest in some aspect of systematic botany, preferably in experimental taxonomy of vascular plants or paleobotany) IN BOTANY—The Secretary and Registrar, The University, Southampton (February 1).

LECTURER (with a good university degree and experienced in the teaching of physics and/or mathematics in schools) IN EDUCATION—The Secretary and Registrar, The University, Southampton (February 1).

CHAIR OF PHYSIOLOGY IN THE LONDON HOSPITAL MEDICAL COLLEGE—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 1).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN PHYSICS, CHEMISTRY, BIOCHEMISTRY, ENGINEERING, METALLURGY AND PHARMACOLOGY—The Registrar, The University, Liverpool (February 13).

JUNIOR LECTURERS (2) IN MATHEMATICS—Prof. E. C. Titchmarsh, Mathematical Institute, The University, Oxford (February 20).

ENTOMOLOGIST (with a good honours degree in zoology or entomology and preferably postgraduate research experience in forest or timber entomology) with the West African Timber Borer Research Unit, Kumasi, Ghana, to undertake studies of the biology, ecology and control of timber and lumber pests—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD 197/510/01T.

REPORTS and other PUBLICATIONS

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Becoming a Teacher. Pp. 8. (London: Ministry of Education, 1959.) [411

Imperial College of Science and Technology (University of London). Prospectus 1960–61. Pp. viii+56+4 plates. Calendar, 1959–60. Pp. xi+378. (London: Imperial College of Science and Technology, 1959.) [411

The Hampstead Scientific Society. Report and Record for 1953 to 1959, With a List of Members. Pp. 24. (London: Hampstead Scientific Society, 1959.) [411

Annual Report on the Meteorological Office for the year April 1, 1958, to March 31, 1959. Pp. 51+5 plates. (M.O. 659.) (London: H.M. Stationery Office, 1959.) 3*s.* 6*d.* net. [411

Great Britain and Ireland

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LETTERS TO THE EDITORS

CHEMISTRY

Influence of Crystal Defects on the Szilard-Chalmers Process in Solids

EVEN if care is taken to ensure constant conditions of irradiation, both with respect to the dosage of fast and slow neutrons, as well as ionizing radiation and heat, and if the post-irradiation treatments are exactly the same; the reproducibility of measurements of the retention of activity in the chemical state of the target molecules, following thermal neutron capture in solids, is generally less than the measurement techniques employed would lead one to expect.

A variety of experimental evidence has now been obtained that indicates that the density, and possibly the nature, of the crystal defects in the irradiated material influence the retention, as well as the kinetic parameters of the subsequent thermal annealing of the recoil damage. The irreproducibility remarked above may well reflect these effects.

It has already been reported that the defects generated by pile neutron irradiation accelerate the post-irradiation thermal annealing of the recoil damage in potassium chromate[1] and ferrocene[2]. A brief statement of some new observations on these effects follows.

Measurements have been made on samples of single crystals and powdered potassium chromate prepared from the same batch of crystals and subjected to thermal neutron irradiation. In contrast to some earlier measurements[3], the value of this retention before any annealing, R_0, was greater for the single crystals (77·3 per cent) than for the powder (69·9 per cent), although all the conditions of irradiation were the same for the two samples. The powder, however, underwent a much more rapid thermal annealing than the single crystals. Thus, six hours heating at 212° increased the retention of the single crystals by 10·4 per cent, but the corresponding increase for the powder was 19·8 per cent. Crushing the single crystals after irradiation had a negligible effect on the retention.

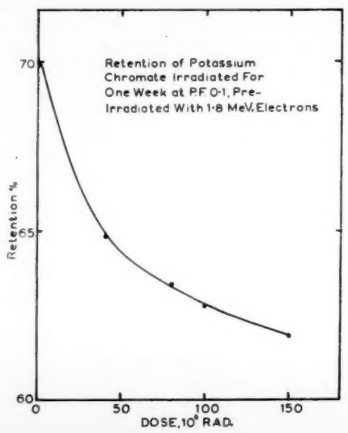

Fig. 1

Pre-irradiation of the single crystals with 1·8-MeV. electrons proved to have much the same effect as crushing the crystals. The change in R_0 with the dose of electrons during pre-irradiation, for constant conditions during and after neutron irradiation, is shown in Fig. 1.

A comparison has been made of the retention and thermal annealing of samples of normal lithium chromate with a sample containing only lithium-7. The samples were prepared and irradiated under the same conditions. They differed only in the isotopic composition of the lithium. The results are shown in Fig. 2.

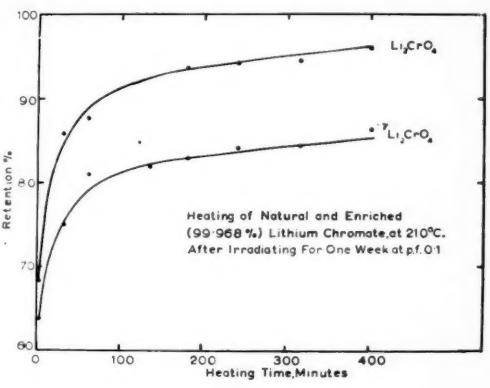

Fig. 2

In this case it appears that the difference in behaviour is to be attributed largely to the much greater radiation damage produced in the crystals containing some lithium-6 because of the fission of this nucleus by the neutrons. A simple calculation shows that the dose arising from this cause amounts to at least as much again as the effects of the fast neutron and gamma radiation.

It is believed that this evidence clearly establishes a relation between the crystal defects or inactive damage centres in the crystal and the behaviour of the recoil fragments.

One of us (J.I.V.) wishes to acknowledge leave of absence from Instituto de Pesquisas Radioativas and the Facultade de Filosofia, University of Minas Gerais, Brazil.

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Annealing of Recoil Damage by Ultra-violet Light

Annealing of the chemical damage caused by thermal neutrons is well known for ionizing radiations such as γ-rays and high-energy electrons[1,2]. However, only one report of annealing by ultra-violet light is recorded in the literature. W. Herr[3] found that the yield of rhenium (VII) from potassium chlororhenite irradiated with neutrons decreased more by heating at 100°C. and irradiating with a mercury ultra-violet lamp than by heating alone. No attempt was made to determine the annealing kinetics or to relate the process to annealing by ionizing radiations. Harbottle[4] reported that four hours bright sunlight had no influence on the retention of potassium chromate.

We have observed the recombination of recoil fragments by ultra-violet light in ammonium dihydrogen phosphate, and have made a comparison of this with high-energy electron annealing. The ammonium dihydrogen phosphate crystals were irradiated with thermal neutrons in *Bepo* at Harwell for 6 hr. at p.f. 0·1. Annealing was carried out using a Hanovia *U.V.S.* 250 mercury lamp, giving a dose of $4{\cdot}5 \times 10^8$ ergs. cm.$^{-2}$ hr.$^{-1}$ at 20 cm., and using 1·8-MeV. electrons from a Van der Graaf accelerator. In both cases care was taken to ensure that the temperature of the samples did not rise above 30°C. Typical plots of the nonphosphate fraction versus dose are given in Fig. 1. The phosphate activity was separated by precipitation of magnesium ammonium phosphate. Although it is not yet possible to compare quantitatively the dose-rate by each annealing method, calculation of the energy from the ultra-violet source incident on the sample shows that the dose-rate cannot be greater than the equivalent of 1 *M* rads/hr. and is probably much less.

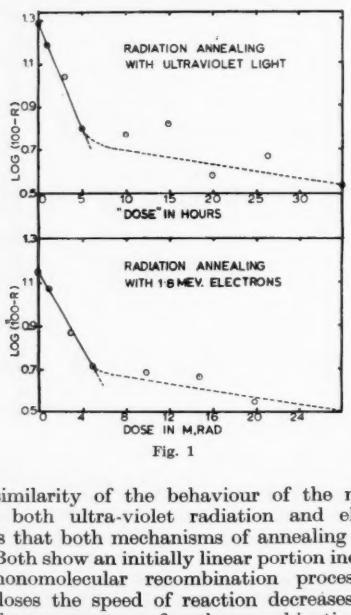

Fig. 1

The similarity of the behaviour of the material towards both ultra-violet radiation and electrons indicates that both mechanisms of annealing are the same. Both show an initially linear portion indicative of a monomolecular recombination process. At higher doses the speed of reaction decreases and a much slower process of unknown kinetics takes over when the retention reaches 95 per cent. The sensitivity of the material to ultra-violet light can be taken as evidence that radiation annealing occurs by an electronic process probably controlled by the defect concentration in the crystal lattice.

Further investigation of the sensitivity of the material to the wave-length of the radiation used is in progress and will be reported in full later.

We are indebted to Tube Investments Ltd., Hinxton Hall, Cambridgeshire, for assistance with the electron irradiations.

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A Correlation between the Shift in the Ultra-Violet Absorption Maxima and the Electronegativity of the Adjacent Atoms in some Aliphatic Carbonyl Compounds

During the course of our recent work on the photochemistry of butyric acid and its methyl ester[1], some spectroscopic confirmation was sought that their absorption spectra and those of the aldehydes and ketones are due to the same electronic transition. The absorption spectra are similar in form and have comparable extinction coefficients, but the absorption maxima of the acids and esters are displaced to lower wave-lengths, as column 2, Table 1, illustrates. There is little

Table 1

1 Compound	2 Wave-length of absorption maximum in A.	3 Reference	4 Shift (wave numbers)	Electro-negativity
Acetaldehyde	2930	8	0	4·6
Acetic acid	2040	9	14930	6·0
Acetone (in water)	2645	8	3730	5·0
Acetone (in hexane)	2790	8	1750	5·0
Acetyl chloride	2345	10, 11	8550	5·5
Acetyl bromide	2400–2600*	10	5910–7570	5·3
Butyric acid	2050	10	14690	6·0
Dimethyl carbonate	1700–1800*	10	21460–24470	7·0
Ethyl acetate	2100	10	13520	6·0
Formaldehyde (in ether)	3150†	12	−1910	4·2

* Estimated range: measurements not extended to maximum.
† Estimated from maximum intensity of partially banded—partly continuous spectrum in reference.

doubt that they are due to the same transition, for the primary photochemical reactions are similar and the carbonyl group in the acids maintains its identity, although it is naturally modified by the neighbouring hydroxyl group[2].

According to Mulliken[3] and McMurray[4], the low extinction of the long-wave absorption of the aldehydes indicates a transition which is forbidden by local symmetry. The spectrum is due to the excitation of an electron from a non-bonding orbital, localized near the oxygen atom of the carbonyl group, to an anti-bonding orbital, spread out over a larger part of the molecule[5]. The upper electronic state is not repulsive, but internal transitions from it to other excited states which are repulsive, lead to the observed photochemical decomposition[6]. The shift in the absorption maximum in going from an aldehyde to an acid may be regarded as an effect of the adjacent atoms to the carbonyl group on the binding of the non-bonding electrons. They will be attracted more by the oxygen atom of the acid than by the hydrogen of the aldehyde and so more tightly bound. Consequently,

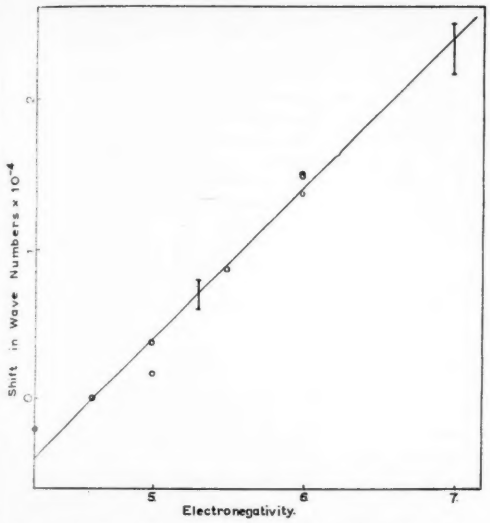

Fig. 1. Relation between shift in absorption maxima and the electronegativity of the adjacent atoms

there will be a relation between the shift in the absorption maximum and the electronegativity of the adjacent atoms. In column 4 of Table 1, the shift in the absorption maximum from that of acetaldehyde is recorded in wave numbers and the final column shows the electronegativity of the adjacent atoms, from Pauling's Scale[7]. The graph plotted from the data, shows a direct relationship between the shift and the electronegativity. The equation of the line is:

$$\Delta\bar{\nu} = [10120e - 47{,}035] \text{ wave numbers,}$$

where e is the electronegativity.

It was found that acetamide and urea fell far above the line, probably due to some delocalization of the electrons, which results in tighter binding and possibly a different transition.

The graph provides spectroscopic evidence that the long-wave absorption maxima in a variety of carbonyl compounds is due to the same electronic transition, and that the orbitals of the carbonyl group have the highest energy in all these compounds. The photochemistry should consequently be similar.

It is also interesting to note that the electronegativity scale has some definite quantitative significance, and may represent an approach to the long-range perturbation of the orbitals in these large compounds which have hitherto been inaccessible to calculation.

I wish to thank Prof. R. G. W. Norrish for his help and encouragement, and the Department of Scientific and Industrial Research for the award of a maintenance grant.

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A Fractional Micro-sublimation Technique

The separation or purification of aromatic hydrocarbons with high melting points is generally carried out by recrystallization, sublimation or absorption chromatography. Purification by zone melting has also been used for substances available in quantity. Absorption chromatography using activated alumina has been used extensively for separating aromatic hydrocarbons. However, it has been found in this laboratory that some substances were difficult to separate by this technique and single bands taken from the column were found in many cases to contain traces of impurities. The detection and separation of these contaminants has been achieved by a simple micro-sublimation technique.

Standard sublimation methods fail to separate traces of substances from a large excess of material, since impurities with low vapour pressures dissolved in the crystal of the bulk material must be held in until the bulk vapourizes and carries across these impurities. The fractional sublimation techniques described by Hausman[1] and Thomas *et al.*[2] both appear to suffer from this defect.

The following simple method seems to be free from this objection and has proved very successful in purifying small quantities of fluorescent aromatic hydrocarbons. A glass tube, 4 mm. internal diameter, was closely fitted into a lagged copper tube C and a temperature gradient (Fig. 1 A) set up along the tube by heating it electrically at one end (Fig. 1 B). A quantity of the sample (maximum of about 15 mgm.) was placed behind a plug of glass wool W in the hot end of the glass tube and the various substances eluted by slowly passing nitrogen (about 10 ml./min.) from the hot to the cold end. The components moved along the tube at different rates depending on their vapour pressures. Two to six hours was required for the full development of the column. The leading edges of the bands were somewhat diffuse and the trailing edges were very sharp. Since a band slowly moves along the tube by continually vapourizing and crystallizing, the impurities with higher vapour pressures move farther along the tube, resulting in a complete separation of impurities from the main band.

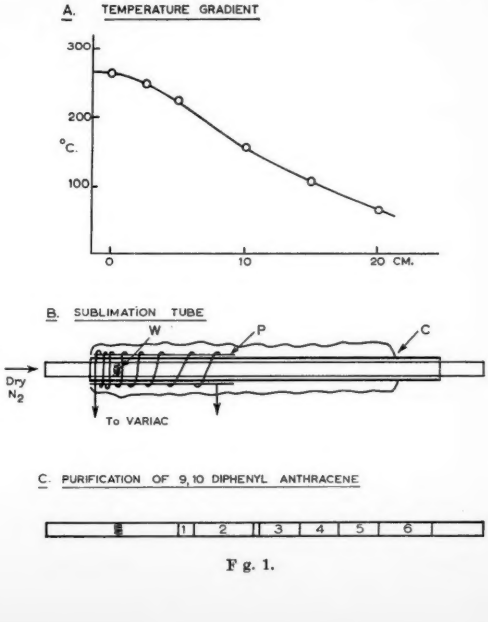

Fig. 1.

Table 1. Separation of Impurities by Fractional Micro-sublimation

Substance	Position in tube (cm.)	Approx. weight (mgm.)
9 Bromoanthracene	3·5– 8	9·5
(unknown)	8–14	Trace possibly 9,10 dibromoanthracene
9 Bromoanthracene	14–19	0·009
Anthracene		0·006
Perylene	3–10	15
Perylene	10–16	0·013
1,2′ dinaphthyl 2,2′ oxide		0·015
Di-2-naphthol	16–21	trace (<0·005)

The separation of bands with different fluorescent colours was most striking in the case of 9, 10 diphenylanthracene which had previously been purified by chromatography on alumina. At least six bands were detected (Fig. 1 *C*) with the following fluorescent colours (~365 mμ exciting radiation): 1=green, 2=blue (16 mgm. of 9,10 diphenylanthracene), 3=bluish green, 4=red (trace), 5=light blue (trace), 6=blue. Two bands, 4 and 6, were present in sufficient quantities to give some light absorption; 4 had peaks at 320 and 247 mμ (ratio of peak heights, 1 to 3) and 6 had a broad band at about 250 mμ.

One disadvantage of this technique when used for the quantitative estimation of substances was the tendency for the bands to overlap, that is, the diffuse leading edge of one band merged into the following one, if the bands were not well spaced on the column. In such cases, the overlapping region was cut out, the substances dissolved in benzene, the solution taken up on glass wool and re-sublimed.

Many substances purified by chromatography on alumina followed by low-pressure sublimation were subjected to this new sublimation technique. In nearly all cases a number of bands were found along the tube, sometimes in sufficient quantities to allow an estimation of their amounts by spectrophotometry (Table 1). Two substances which could not be separated by chromatography were perylene and 1,1′ dinaphthyl 2,8′–2′,8 di-oxide (a possible by-product in the synthesis of perylene). These two substances were mixed and developed on the tube. Both moved at the same rate but formed distinctive crystals which could be readily identified (perylene—flat plates; 1,1′ dinaphthyl derivative—long needles). Only the well-known compound 1,1′ dinaphthyl 2,2′ oxide was found in the perylene synthesized in this laboratory (Table 1).

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BIOCHEMISTRY

A Function for the Extracellular Polysaccharide of *Azotobacter vinelandii*

Recent studies on the nitrogen-fixing pseudomonads and their polysaccharides[1,2] have refocused attention on the function of these extra-cellular products. Beijerinck[3] observed the disappearance of a mucus produced by *Bacillus polymyxa*, and recently the pseudomonads have been shown to degrade their extra-cellular polysaccharide[4], making it plausible that these materials may serve as carbon and energy reserves. Since no definitive experiments on the usefulness of an extra-cellular polysaccharide to the organism are reported to date the following preliminary study was made.

An organism was isolated from aerobic enrichments of soil in a nitrogen-free mineral salts medium containing ethanol as sole carbon scource. The pure culture obtained after repeated streaking was characterized as an *Azotobacter vinelandii* type[5]. Typical growth stages observed on the above medium solidified with agar were: (*a*) at 48 hr. the colony was 2–3 mm. in diameter, raised, translucent, mucoid, with an entire circular edge and smooth glistening surface, (*b*) on the third day after inoculation an opaque zone was observed in the medium below the colony and extending a little beyond the perimeter, (*c*) within an additional 48 hr. the colony collapsed to a flattened growth surrounded by the opaque zone, and subsequently (*d*) a clear zone appeared around the colony. To exclude the possibility that cell lysis had been followed by the outgrowth of a contaminating organism, stage (*d*) colonies were restreaked onto a variety of media but showed only the original isolate. Thus it may be concluded that the phenomenon described is the result of the activities of a single microbial type.

To prepare a sample of the polysaccharide, organisms in stage (*a*) were harvested and washed by centrifugation. The water-soluble material was precipitated from the supernatant by addition of an equal volume of cold acetone. Repeated solution in distilled water and reprecipitations with acetone, followed by air drying, yielded an off-white product. This material gave a positive reaction for carbohydrates, a trace reaction in the biuret test, and dissolved to give an opalescent aqueous solution. The aqueous solution was used in the experiments reported below.

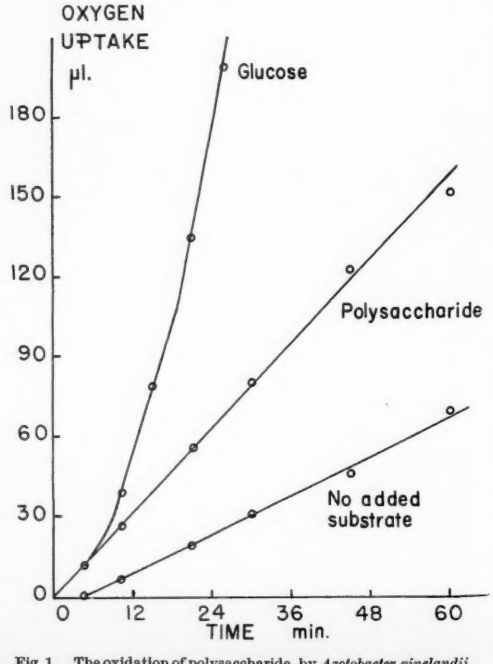

Fig. 1. The oxidation of polysaccharide by *Azotobacter vinelandii* in late growth on ethanol medium (stage (*d*))

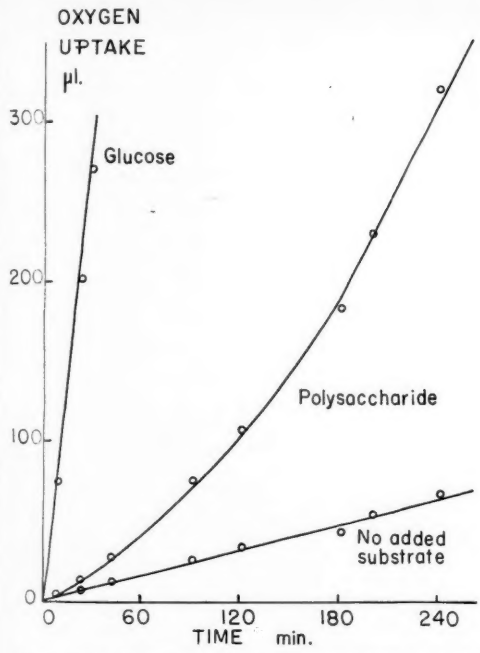

Fig. 2. The oxidation of polysaccharide by *Azotobacter vinelandii* in early growth on ethanol medium (stage (*a*))

Nitrogen-free mineral salts medium solidified with agar was prepared: without added carbon source; with a trace of acetone as carbon source; with ethanol as carbon source; and with polysaccharide as carbon source. Periodic transfers were made from the ethanol medium to these media with the following results. Cells from stage (*a*) grew rapidly on ethanol, and after a lag period of approximately 24 hr. on the polysaccharide medium. Cells from stages (*c*) and (*d*) grew rapidly and luxuriantly on both ethanol and polysaccharide, and produced clearing of the initially opaque polysaccharide medium. Cells from any growth stage grew to a negligible extent on the control media.

Oxidation of the polysaccharide was studied with standard manometric technique. After harvest from the surface of the ethanol medium, the cells were washed by centrifugation and resuspended in 0·01 *M* phosphate buffer *p*H 7·0, prior to aeration before use. The oxygen uptake from a gas phase of air was observed with polysaccharide as substrate. Controls with glucose were included to indicate the general physiological state of the cells. The results in Fig. 1 show that the organisms in stage (*d*) are able to metabolize the polysaccharide without lag at a rate significantly above the endogenous; while the results in Fig. 2 show that organisms in stage (*a*) are also able to metabolize the polysaccharide, but only after a lag period. Cultures grown with polysaccharide as carbon source metabolize as stage (*d*) organisms.

All these observations are consistent with the postulate that the organisms are able to use their extra-cellular polysaccharide as a carbon and energy source. Thus the polysaccharide may be regarded as a true storage material.

These experiments were carried out at the Hopkins Marine Station, Pacific Grove, California, and I would like to thank Dr. C. B. van Niel for the hospitality of his laboratory.

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Occurrence of Leuco-Anthocyanin in Sour and Sweet Cherries

In recent years there has been an increasing interest in the role of leuco-anthocyanins as possible precursors of anthocyanins *in vivo*. Evidence for and against this hypothesis has been presented by many workers, and reviewed by Alston[1]. In the course of the isolation and identification of the anthocyanin pigments of sour cherries[2] and sweet cherries[3] in this Laboratory, these fruits at two stages of maturity were examined for the occurrence of leuco-anthocyanins. Evidence was found for the presence of the leuco-anthocyanin related to cyanidin, which is also the single nucleus for the anthocyanin pigments in cherries[2,3].

Green and yellow fruits of sour cherry (*Prunus cerasus* L., var. Montmorency) and two varieties of sweet cherry (*Prunus avium* L.), the Windsor and Yellow Glass, were found to have leuco-anthocyanins in both the peels and flesh by a method[4] of detection involving heating the plant tissues in 2 *N* hydrochloric acid at the temperature of boiling water for 20 min., and extracting the red anthocyanidin pigment so formed with iso-amyl alcohol. This derived pigment was shown to consist solely of cyanidin in all cases by paper chromatography, colour reactions and absorption spectra, in comparison with authentic cyanidin, as previously described[2,3].

It is to be noted that the amount of cyanidin, derived from the leuco-anthocyanin, was 5–6 times as much as obtained from sour cherries, than from sweet cherries at a similar stage of maturity. The yield of cyanidin from the two varieties of sweet cherries was about the same. The concentration of cyanidin in the acidified *iso*-amyl alcohol solution was estimated by the optical density of absorption at 550 mμ. As the cherry fruits matured, such amounts of cyanidin decreased in all cases. However, an interesting fact is that the colour of the fruits of the Yellow Glass variety of sweet cherry, unlike the Windsor variety, is yellow though fully ripened.

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Inhibition of Urease by Alloxan and Alloxanic Acid

Ever since the discovery that alloxan could induce diabetes[1] in animals, this compound has been the agent of choice in producing experimental diabetes. While the maximal modifications in the chemical structure of alloxan compatible with maintenance of diabetogenic action are known, its mode of action is still debated. That property of alloxan most frequently implicated in studies on its action is its ability to inhibit a variety of -SH enzymes such as succinoxidase, urease, papain, cathepsin, and the enzyme which acetylates aromatic amines[2], although its action is not confined to such enzymes.

The purpose of this communication is to present evidence implicating alloxanic acid, a non-diabetogenic compound which is the spontaneous breakdown product of alloxan at physiological *p*H's, as an inhibitor of an -SH enzyme, urease.

In the course of studies on the degradation of alloxan and alloxanic acid by extracts of a soil bacterium, it was found that glyoxylate and urea tended to accumulate. Glyoxylate accumulation appeared to be due to a deficiency in thiamine pyrophosphate; but the persistence of urea was difficult to reconcile with the presence of a potent urease in the extracts. It seemed possible that alloxan, alloxanic acid, or certain of their breakdown products were inhibiting the urease, and indeed it was found that alloxanic acid is a strong inhibitor of bacterial urease. These studies on the inhibitory effect of alloxanic acid on bacterial urease were done at *p*H 7·0. Alloxan is unstable[3] at *p*H's above 3·5 and has a half-life of only a few minutes at 7·0 and less than a minute at 7·4, before it is converted to alloxanic acid, and therefore could not be tested at this *p*H. In as much as alloxan has been reported to inhibit various sulphydryl enzymes, and alloxanic acid in our hands was a potent inhibitor of bacterial urease, it seemed possible that alloxan inhibition of an isolated enzyme system might in reality be due to the action of the spontaneous decomposition product. Urease is an -SH enzyme which still possesses considerable activity at low *p*H's where alloxan is relatively stable, and so it was possible to compare alloxan and alloxanic acid as inhibitors.

Studies employing crude and crystalline jack bean urease, which were easier to obtain in quantity and more stable than bacterial urease, were carried out at *p*H 5·0 in acetate buffer[4]. Fig. 1 shows that alloxanic acid is a potent inhibitor of urease, whereas alloxan shows little inhibitory effect until a period has elapsed during which some alloxan is converted to alloxanic acid. When the experiment is done at *p*H 7·0 the inhibitory action of alloxan and alloxanic acid is identical, as at this higher *p*H some of the former compound is immediately converted to the latter. As one might postulate, it is possible to overcome the inhibition with glutathione or cysteine. Kinetic studies show that the inhibition is essentially, but not purely, non-competitive. Perhaps the presence of the urea moiety in the inhibitor superimposes a slight competitive element on the inhibition. By varying the concentration of alloxan and alloxanic acid and extrapolating the inhibition observed at frequent intervals to zero time, it was shown that alloxanic acid is fifteen to twenty times more effective than alloxan as an inhibitor.

A number of compounds structurally related to alloxan and alloxanic acid were tested for their ability to inhibit jack bean urease (Table 1). Alloxan,

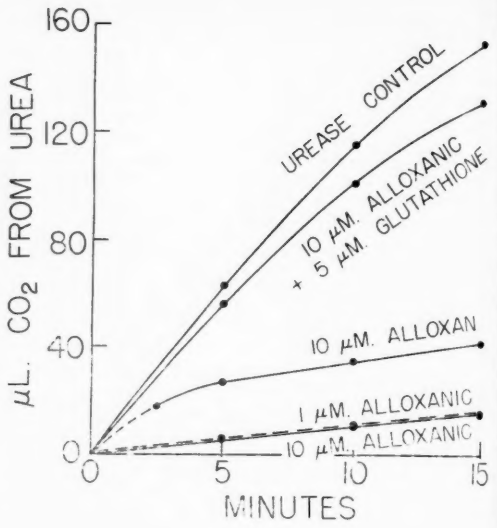

Fig. 1

alloxanic acid, dialuric acid, alloxantin, and murexide are powerful inhibitors, and each contains either the alloxan or alloxanic acid moiety. Alloxan compares favourably with alloxanic acid in these experiments because inhibition was determined over a 20-min. period which permitted gross formation of alloxanic acid. Alloxantin, a diabetogenic compound, is composed of a molecule of alloxan and another of dialuric acid; murexide, a non-diabetogenic compound, contains alloxan plus uramil. Dialuric acid, which is spontaneously oxidized to alloxan and then converted to alloxanic acid, was also inhibitory. Siliprandi and Daghetta[2] previously had found that dialuric acid did not inhibit urease when the reaction was carried out under nitrogen. Under conditions which do not permit oxidation dialuric acid is not a diabetogenic compound.

There is little doubt that sulphydryl enzymes interact with alloxan and that -SH compounds influence that action of alloxan. The prevention of alloxan diabetes by administration of glutathione or cysteine[5] and the increased susceptibility of animals on a diet deficient in sulphur-containing amino-acids to the diabetogenic action of alloxan and the relatively non-diabetogenic compound, uric acid[6], are known.

Table 1. Effect of alloxan, alloxanic acid and other compounds on the action of urease

Compound	Molarity	Inhibition (per cent)
Alloxan	0·01	72
	0·001	62
Alloxanic acid	0·01	88
	0·001	85
Dialuric acid*	0·01	89
	0·001	40
Alloxantin*	0·01	97
	0·001	84
Murexide	0·01	94
	0·001	62
Uric acid*	0·01	17
Allantoin	0·01	9
5-OH hydantoin	0·01	5
Hydantoic acid	0·01	14
Hydantoin	0·01	3
Parabanic acid	0·01	0
Oxaluric acid	0·01	3
Oxalic acid	0·01	0

* Not completely soluble at 0·01*M*.

The test system consisted of 10 μmol. urea, 0·2 mgm. jack bean urease protein, 0·09 *M* acetate buffer *p*H 5·0, and test compound in a total volume of 1·1 ml. Incubation was for 20 min. in Warburg vessels in air at 30°C.

However, it would seem that structural considerations other than -SH binding capacity are involved in alloxan action since the closely related, but non-diabetogenic compound, alloxanic acid, is the more potent -SH inhibitor. If one accepts the figures given for the rapid conversion of alloxan to alloxanic acid in blood plasma[7] as representing the rate of alloxan breakdown after intravenous injection, then the animal contains primarily alloxanic acid in a matter of minutes. It is logical to suppose that a certain portion of the alloxan exerts its action before this conversion is complete. The same possibility exists with regard to the action of alloxan on isolated enzyme systems. It is, however, difficult to interpret many of the early *in vitro* studies because they were carried out at neutral *p*H's where alloxanic acid could have been responsible for the inhibition observed. While the introduction of controls in the form of aged neutral solutions of alloxan has given validity to certain recent studies, there has been no attempt to assign any background inhibition to a specific degradation compound such as alloxanic acid. The possibility that alloxanic acid may have a synergistic action by creating an -SH deficiency exists.

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Paper Chromatography of Corticosteroids at Room Temperature

THE numerous paper chromatographic methods[1-3] so far presented for the separation of corticosteroids all have limitations; our results may therefore be of interest. We have found that by impregnating the papers with a 90 per cent methanol solution we could separate the corticosteroids rapidly at room temperature (16–20°C.). The method also works for prednisone and prednisolone (Table 1). Furthermore, it is suitable for quantitative and semi-quantitative determinations.

1–200 μgm of the steroids, dissolved in methanol, was spotted on to strips 2 cm. wide prepared from 45 cm. × 4 or 15 cm. Schleicher-Schüll 2043/*b* papers. The strips were then impregnated by drawing them through a 90 per cent methanol solution, leaving a dry space, 0·5–1 cm. wide, at the starting line. The excess liquid was pressed out between two layers of filter paper and the strips were immediately placed in the apparatus.

Two sizes of glass chamber were employed, depending on the size of the paper. Ten minutes before use, porcelain cups containing wads of paper were placed in each chamber. One cup was filled with the organic phase, the other with 90 per cent methanol solution. This served to saturate the atmosphere in the chambers. The small chambers were also lined with a piece of filter paper 20 cm. × 20 cm. saturated with the aqueous phase.

Table 1. R_F OF CERTAIN STEROIDS AFTER IMPREGNATION IN 90 PER CENT METHANOL

Steroid	Solvent System*							
	1		2		3	4	5	6
	R_F	R_FC†	R_F	R_FC†		R_F		
Hydrocortisone	0·24	0·55	0·09	0·46	0·12	0·39	0·16	0·49
Aldosterone	0·34	0·80	0·13	0·71				
Cortisone	0·40	1·0	0·17	1·0	0·20	0·56	0·37	0·64
Corticosterone	0·60		0·48		0·51	0·74	0·71	0·80
Reichstein's *S* compound	0·63		0·49		0·54	0·76	0·75	0·84
11-dehydro-corticosterone	0·68		0·66		0·67	0·85	0·84	0·90
11-deoxycorti-costerone	0·81		0·90		0·85	0·96	0·98	0·98
Prednisolone	0·20	0·50	0·05	0·25				
Prednisone	0·34	0·82	0·13	0·72				

* Solvent mixture: 1, toluene-ethanol-petroleum ether-water (2:6:1:3); 2, Bush system[4] toluene-methanol-water (10:7:3); 3, Bush[4] *B* 2 system; 4, Pechet[3] No. 1; 5 Pechet No. 2; 6 Pechet No. 6.
† R_F values related to cortisone after 16 hours of development.

The organic and aqueous phases were prepared from a mixture of toluene-ethanol-petroleum ether-water (2:6:1:3). The mixture was allowed to stand for 18 hours and after separation the organic phase was filtered.

Development was carried out for 2–2·5 hr., during which time the front sank 30 cm. The cylinder was covered with a glass plate and the top and bottom were sealed with a glycerine-starch paste.

Under similar experimental conditions good separations were obtained with Bush's[4] solvent mixture (toluene-methanol-water, 10:7:3), Bush's *B*2 system, and Pechet's[3] solvents, Nos. 1, 3 and 6.

An alkaline solution of tetrazolium blue and 15 per cent phosphoric acid were most frequently used for detection.

Further experiments are in progress.

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Lability of Fibrinolysin

FEARNLEY'S method for estimating fibrinolytic activity of normal blood[1] is based on the fact that the clot formed by adding thrombin to blood diluted 1 : 10 with phosphate buffer will lyse on incubation. This lytic activity may be lost if the specimen is permitted to stand for hours or even minutes at room temperature. Thus Fearnley and others caution that specimens must be kept in an ice bath and preferably collected in pre-chilled vessels. In contrast, the techniques used in our studies of fibrinolysis induced by nicotinic acid[2] employing essentially undiluted plasma and the 'thrombelastograph'[3] fail to show spontaneous lysis of normal plasma. The lytic activity induced by parenterally administered nicotinic acid is clearly demonstrable by our methods, and is not destroyed by many hours storage at room temperature or even two weeks at 5° C.

When a 10 : 1 phosphate-diluted normal blood specimen is observed in the thrombelastograph, the

recording shows the development of a clot of only slight firmness, followed by a typical pattern of gradual lysis, well correlated in time with the test-tube observation of lysis. This confirms that the differences observed by Fearnleys' technique as contrasted to ours, result from the nature of the system rather than the mechanics by which lysis is observed.

In our first attempt to duplicate Dr. Fearnley's technique, our normal specimens regularly showed lysis whether or not we used ice bath precautions in obtaining and handling them. We suspected that varying the molarity and nature of diluent might influence the stability of fibrinolysin.

Two aliquots of the same blood specimen were stored for 1½ hr. at 0° C. and 37° C. respectively. One tenth ml. aliquots of each of these were then added to 0·9 ml. of phosphate buffer in 11 different concentrations ranging from 0 to 0·075 *M*, and to similar sodium chloride solutions.

Table 1, indicating complete (++), incomplete (+), or no evidence of lysis (0) after 24-hr. incubation, demonstrates that both molarity of diluent and temperature of storage influence lysis. With higher ionic strength of the diluents, lysis occurs regularly even after incubation of the specimen.

Table 1

mMols./l.	..	0	8	15	23	30	38	45	52	60	68	75
PO_4 0°	..	+	+	+	+	++	++	++	++	++	++	++
PO_4 37°	..	0	0	0	0	0	+	+	+	++	++	++
NaCl 0°	..	+	+	+	+	++	++	++	++	++	++	++
NaCl 37°	..	0	0	0	0	0	0	+	+	++	++	++

These results are consistent with the report by Buckell and Truscott[4] which has come to our attention since the above experiments were conducted. It remains to be determined whether hypertonic diluents may not simplify or increase the sensitivity of fibrinolysin estimating techniques. It also remains to be determined to what extent the relatively stable activated fibrinolysin detected in essentially undiluted systems represents a different moiety from that detected by dilution.

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Change of Absorption Spectrum of Flavin Adenine Dinucleotide byits Binding with both D-Amino Acid Oxidase Apo-Protein and Benzoate

Demonstration of the actual binding of apo-protein, coenzyme and substrate of D-amino acid oxidase is necessary in order to resolve the processes involved in the dynamic enzyme complex formation resulting in the manifestation of chemical reaction catalyzed by this enzyme. Therefore, we have examined the change of absorption spectrum of flavin adenine dinucleotide by D-amino acid oxidase apo-protein and the substrate. D-Amino acid oxidase apo-protein and flavin adenine dinucleotide were prepared by the methods of Negelein, Brömel[1] and Yagi *et al.*[2], respectively.

The change of absorption spectrum of flavin adenine dinucleotide by combining with the apo-protein was measured by the difference spectrum for the apo-protein ($2{\cdot}3\times10^{-5}$ mole/l.) before and after the addition of flavin adenine dinucleotide ($7{\cdot}7\times10^{-6}$ mole/l.) in pyrophospate buffer (*p*H 8·3, *M*/10). The absorption spectrum of flavin adenine dinucleotide in the visual light region obtained by the above-mentioned procedure was suppressed and shifted slightly to a longer wave-length region compared with its original absorption spectrum. The maxima of absorption spectrum of flavin adenine dinucleotide at 375 mμ and 450 mμ were shifted to 377 mμ and 453 mμ respectively, by combining with the apo-protein as shown in curve II of Fig. 1. These results nearly agreed with those reported by Negelein and Brömel[1]. The absorption spectrum of flavin adenine dinucleotide in the ultra-violet region was suppressed and shifted from 263 mμ to 265 mμ. These results nearly agreed with those reported by Kubo[3].

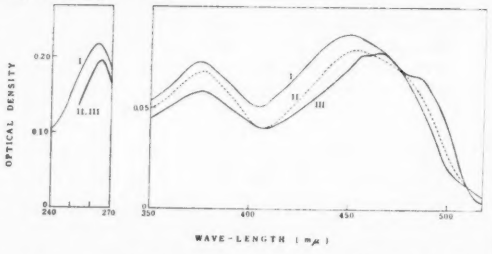

Fig. 1. Change of absorption spectrum of flavin adenine dinucleotide by combining with both D-amino-acid oxidase apo-protein and benzoate.
Curve I, absorption spectrum of flavin adenine dinucleotide $7{\cdot}7\times10^{-6}$ mole/l.) in pyrophosphate buffer (*p*H 8·3, *M*/10); Curve II, difference spectrum of D-amino-acid oxidase apo-protein ($2{\cdot}3\times10^{-5}$ mole/l.) before and after the addition of flavin adenine dinucleotide ($7{\cdot}7\times10^{-6}$ mole/l.) in the same buffer; Curve III, difference spectrum of D-amino-acid oxidase apo-protein before and after the addition of both flavin adenine dinucleotide ($7{\cdot}7\times10^{-6}$ mole/l.) and benzoate (1×10^{-4} mole/l.) in the same buffer

Then the influence of the substrate on the above-mentioned absorption spectrum of flavin adenine dinucleotide was studied by using a substitute for the substrate which does not reduce the co-enzyme. For this purpose, benzoate was selected. The reasons for the selection was that benzoate competes with the substrate for the binding sites of the enzyme[4,5], and that benzoate does not change the absorption spectrum of flavin adenine dinucleotide when it is mixed with the latter in aqueous solution.

The difference spectrum for the apo-protein before and after the addition of both flavin adenine dinucleotide and benzoate (1×10^{-4} mole/l.) was measured under the same conditions as above. It differs from that of flavin adenine dinucleotide combined with the apo-protein in two important respects: (1) the intensity of the absorption at 450 mμ is further decreased and that at 485 mμ is increased to form a shoulder; (2) a new absorption peak appears at 465 mμ, as shown in curve III of Fig. 1. These changes of the absorption spectrum suggest that the combination of *iso*alloxazine nucleus of the co-enzyme with the apo-protein is increased by benzoate, and that a new bond occurred between benzoate and isoalloxazine nucleus besides that between isoallozazine nucleus and the apo-protein.

In the ultra-violet region, however, the intensity and the shape of the spectrum of flavin adenine dinucleotide combined with the apo-protein was not changed by the co-existence of benzoate. Considering the fact that binding occurs via the adenylic acid part of flavin adenine dinucleotide and a sulph-

ydryl group of the apo-protein as reported in the previous communication[6], the results obtained here suggest that benzoate does not combine with adenylic acid part of flavin adenine dinucleotide.

From these results, it may be concluded that the isoalloxazine nucleus of flavin adenine dinucleotide binds with both the apo-protein and the substrate by its two different binding sites, and that the binding site in adenylic acid part of flavin adenine dinucleotide joins only with the binding site of the apo-protein.

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Occurrence of Polynucleotide Phosphorylase in Atypical Epithelioma of Rat

SINCE the discovery of polynucleotide phosphorylase in *Azotobacter vinelandii*[1,2,3], its wide distribution has been expected concerning its actual meaning in polynucleotide synthesis in living body. Though it has been found to exist in several bacteria[4,5], its occurrence in animal source has not been demonstrated except in human sperm reported by Hakim[6]. In our laboratory, the existence of this enzyme in animal cells has been examined, and the occurrence of it in atypical epithelioma (Guerin strain) was found. The results are briefly reported here.

The tissue of atypical epithelioma of a rat about two weeks after transplantation was removed immediately after decapitation of the animal and was immersed at once in ice-cold 0·9 per cent sodium chloride solution. The supporting connective tissue and areas of necrosis of this epithelioma tissue were carefully removed. Then the tissue was homogenized with four times of its volume of 0·05 *M tris*(hydroxymethyl)aminomethane buffer, *p*H 7·4 at about 0°. The isolation and partial purification of polynucleotide phosphorylase was carried out with this extract of atypical epithelioma according to the method of Littauer and Kornberg[5]: namely, manganese chloride was added to the extract, and the enzyme in the supernatant obtained by the centrifugation of this mixture was adsorbed on protamine sulphate and was eluted. The nucleic acid content of this enzyme preparation was calculated to be 1·5 per cent of protein concentration, from the ratio of optical density at 280 mμ to that of 260 mμ. Then the preparation was further purified by zinc-ethanol fractionation. The preparation obtained by this fractionation was used for enzymic reactions.

The measurements of activity of this enzyme were carried out according to the method of Beers[4]. The enzymic reaction mixtures were composed of each 0·5 ml. of enzyme preparation, 2 m.moles of adenosine-5′-diphosphate, 3 m.moles of magnesium chloride and 0·1 *M tris*(hydroxymethyl)aminomethane buffer, *p*H 10·0. The solution was incubated at 37° for 15 min. Then 2 ml. of 5 per cent (w/v) perchloric acid was added to the reaction mixture and precipitate formed was collected by centrifugation. As controls, the following two experiments were performed: (1) the same reaction mixture as above was added with perchloric acid without incubation; (2) the reaction mixture without adenosine-5′-diphosphate was treated in the same way as the main experiment. The precipitate was washed by 5 per cent perchloric acid (w/v) and then washed twice by 95 per cent ethanol. Finally the sediment was suspended in 0·1 *M tris*(hydroxymethyl)aminomethane buffer, *p*H 8·0. The insoluble denatured enzyme protein was centrifuged off. The absorption spectrum of the supernatant thus obtained which is considered to contain only adenosine-5′-phosphate polymer is shown in Fig. 1. The maximum absorption of this synthesized polymer exists at 260 mμ. The optical densities at 260 mμ measured with the main experiments were found to be 0·5–0·7, whereas those of the controls were less than 0·05.

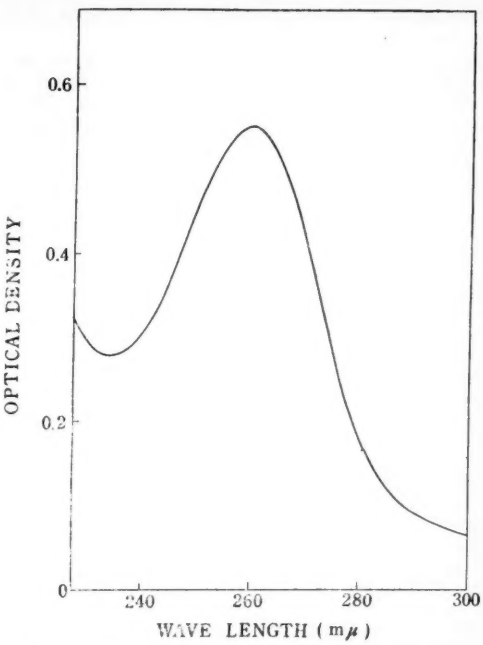

Fig. 1. Absorption spectrum of synthesized adenosine-5′-phosphate polymer catalysed by polynucleotide phosphorylase extracted from atypical epithelioma of rat. The illustrated spectrum is that of the reaction product of polynucleotide phosphorylase at 37° for 15 min

The synthetic activity of polynucleotide phosphorylase found in an epithelioma is of special interest from the point of view of rapid growth of neoplasmatic tissue.

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Cytoplasmic Nucleopeptides in Guinea Pig Liver

Nucleopeptides have been isolated from a variety of biological materials. They have been found in yeasts[1], *Streptococcus fæcalis*[2] and *Chlorella*[4]. There is also some record of the existence of fractions of ribonucleic acid containing peptides in a bound form[3].

In this communication the subfractionation of dialysable nucleopeptides from guinea pig liver is reported and certain properties of the subfractions described.

Ground liver tissue (6 gm.) was homogenized with an equal weight of 0·25 *M* sucrose solution, the dispersion diluted with more sucrose up to ten times the original weight of the tissue, and then centrifuged at 0° and 10,000 *g*. The supernatant was saturated with solid ammonium sulphate, centrifuged in the cold at 32,000 *g*, and the precipitate discarded. The supernatant was treated with cold ethanol to a final concentration of 85 per cent and the precipitated ammonium sulphate removed by centrifugation. The ethanol was then removed by distillation *in vacuo* and the concentrated material passed through a glass filter (Schott *G* 4). From this concentrated solution a sample corresponding to 1·2 mgm. of ribonucleic acid (as estimated by absorption at 260 mμ) was withdrawn, diluted with water to 10 ml. and transferred to a column (1·5 cm. in diameter and 12 cm. long) containing triethylaminoethyl cellulose[5]. The fractionation was carried out by elution at 8–10°, first with 0·01 *M tris* buffer *p*H 7·4, and then with the same buffer but containing increasing concentrations of sodium chloride. Afterwards the column was eluted with 1 *M* sodium chloride and finally with 1 per cent sodium hydroxide. The ribonucleic acid content of the eight fractions thus obtained was followed by measuring the light extinction at 260 mμ. The results are given in Fig. 1.

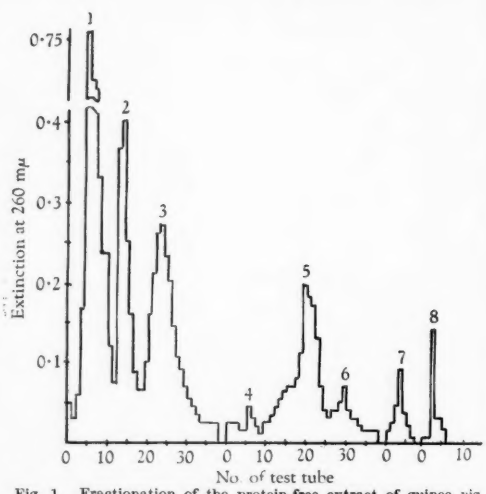

Fig. 1. Fractionation of the protein-free extract of guinea pig liver on a column filled with triethylaminoethylcellulose. Eluents used: fractions 1–3, 0·01 *M tris* buffer, *p*H 7·4; fractions 4–6 0·01 *M tris* buffer containing increasing concentrations of sodium chloride; mixing camer, 0·5l. Fraction 7, 1 *M* sodium chloride solution in 0·01 *M tris* buffer: fraction 8, 1 per cent sodium hydroxide. Period of collecting each fraction: 8–10 min. Vol. of each fraction: 3·8 ml.

All eight fractions were examined for the presence of purine and pyrimidine bases, ribose, amino-acids, and activated amino-acids or peptides. For the identification of purine and pyrimidine bases samples were evaporated to dryness, hydrolysed for 1 hr. at 100° with 12 *N* perchloric acid, and after elimination of perchloric acid subjected to chromatography, using hydrochloric acid:*iso*propanol:water[6] and formic acid: butanol-water[7] as solvents. The presence of adenine, guanine, cytosine, uracil, and thymine was detected in all eight fractions. For the identification of ribose chromatography was used after the samples have been treated with bromine water[8]. Amino-acids were demonstrated by the reaction with ninhydrin[9]; this was positive in all eight fractions. On hydrolysis of the samples it became considerably stronger thus indicating the presence of peptides.

On treatment with salt-free hydroxylamine and acid ferric chloride solution a pink-brown colouration developed in all fractions, due to the formation of hydroxamic acids. The presence of carboxyl-activated amino-acids or peptides was examined further by treating the samples with hydroxylamine and subjecting them to chromatography. After treatment with ferric chloride 6–8 hydroxamate spots were revealed. The spots were excised, eluted with 0·1 hydrochloric acid and the eluates hydrolysed for 16 hr. with 6 *N* hydrochloric acid at 105°. In each spot the following amino-acids identified: cysteine, tyrosine, glycine, glutamic acid, methionine, aspartic acid, histidine, lysine, serine, alanine, valine, phenylalanine and leucine.

Paper electrophoresis carried out in each fraction on Whatman paper No. 1 in 0·1 *M* ammonium acetate buffer *p*H 5·9 at 0°, 15 hr., yielded 2–3 spots which showed violet fluorescence in ultra-violet light. Those spots which moved towards the cathode produced pink-brown colour on treatment with hydroxylamine and ferric chloride solution, thus indicating the presence of carboxyl-activated peptides. The spots moving towards the anode did not develop any colour under the same conditions. Thus it could be seen that the fractions contained some free nucleic substances as well as ones bound to peptides.

The eluates from single cathode-moving spots were treated with hydroxylamine and hydroxamates were chromatographed. After treatment with ferric chloride, 2–4 pink-brown spots were revealed. This result might indicate that one nucleotidic moiety was bound with a couple or more of peptides, providing the homogenity of each electrophoretic spot.

The above results indicate that protein-free extracts from guinea pig liver contain certain oligonucleotides, each attached to 2–4 carboxyl-activated peptides.

The possibility that such complexes may be involved in the protein biosynthesis is now under investigation.

A more detailed account of the experiments will be published in the *Acta Biochimica Polonica*.

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Quantitative Estimation of the Fatty Acids of Olive Oil

BEFORE making a systematic study of the fatty acid composition of Spanish olive oils it was necessary to devise a procedure which combined precision and accuracy with speed. In this brief account we present a summary of the results obtained in our work, involving (1) spectrophotometry and (2) quantitative paper chromatography of the saturated acids.

The spectrophotometric estimation of unsaturated fatty acids is carried out by the method recommended by the American Oil Chemists' Society[1]; saturated acids, separated according to Bertram[2], are chromatographically determined by a procedure recently developed in this laboratory[3]. This procedure is an extension of that of Kaufmann and Nitsch[4]; the paper chromatograms are dipped in cupric acetate solution followed by treatment with rubianic acid to reveal the spots, the area of which is determined microphotometrically on photographs of the chromatograms.

The results of the analysis of a sample of olive oil from the 1957–58 crop in Bujalance (Cordoba) are compared in Table 1 with results on the same oil

Table 1. COMPONENT FATTY ACIDS (PERCENTAGE BY WEIGHT) OF A SAMPLE OF OLIVE OIL AS DETERMINED BY DIFFERENT METHODS

Acid	Distillation of methyl esters	From thiocyanogen value	Method Gas chromatography	Spectrophotometry and paper chromatography
Saturated				
Myristic	0·2		—	—
Palmitic	8·9		11·7	7·7
Stearic	2·4	11·9	2·4	2·5
Arachidic	0·3		—	0·7
Behenic	—		—	0·8
Unsaturated				
Hexadecenoic	4·7		1·2	76·8
Oleic	66·3	75·5	73·5	
Eicosenoic	4·9		—	
Linoleic	12·3	12·6	11·2	10·9
Linolenic	—	—	—	0·6

obtained (*a*) by the traditional ester fractionation method[5], (*b*) from thiocyanogen values and (*c*) by the modern technique of gas chromatography at 170° using polymerized ethylene glycol adipate as liquid phase and argon as carrier gas.

The results obtained by our combination of spectrophotometry and paper chromatography agree very well with those obtained by other methods; the absence of traces of myristic acid in the gas chromatographic analysis is rather surprising, though its absence from the paper chromatograms is not, since any present initially would have been degraded in the Bertram oxidation procedure. The small amount of behenic acid detected by paper chromatography would remain in the ester distillation residues and escape detection and it would also not be apparent in the gas chromatograms under the conditions employed. This acid has previously been identified by Spiteri[6] in olive oil; but the lignoceric acid also reported by him did not appear on our paper chromatograms. Linolenic acid, not revealed by gas chromatography, is apparently present as judged by the spectrophotometric evidence; but this cannot be taken as unequivocal proof of identity.

We feel that the method proposed by us will be of considerable value to workers who, lacking facilities for chromatography, require rapid analysis of olive oil for its main component acids. Unlike the time-consuming ester distillation procedure which requires somewhat more starting material and in which no saturated acid is usually unequivocally identified our method is relatively quick, saturated acids are identified, and the amount of oil required is quite small.

We wish to thank Dr. D. P. Cuthbertson, director of the Rowett Research Institute, Aberdeen, Scotland, for granting laboratory facilities to one of us (E. V.) which permitted the gas chromatographic analysis to be included.

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Interaction between Thiourea and Insect Polyphenoloxidase

POLYPHENOLOXIDASES of plant and animal origin are known to be inhibited by copper complexing sulphur compounds. When investigating the effect of thiourea on polyphenoloxidase of the wax-moth, *Galleria mellonella* L. (Lepidoptera), we noticed that degree of inhibition diminished during an aerobic incubation of the enzyme with the inhibitor and the substrate (catechol or *p*-cresol).

Experiments have been performed with homogenates from whole fully grown larvæ, and a typical course of oxygen uptake in the presence and absence of thiourea is shown in Fig. 1. The rate of uptake of oxygen by the uninhibited sample (indicated by the slope of curve *A*) diminishes steadily with the time of incubation which is a result of the inactivation of polyphenoloxidase[1]. On the other hand, the rates of oxidation in the presence of various concentrations of thiourea (slope of curves *B* and *C*), which are initially low, increase considerably during the incubation, attain a certain maximum and finally drop as result of inactivation of the enzyme. This is better seen in Fig. 2 where the rates of uptake of oxygen are plotted against time of incubation. It is thus apparent that a release of the inhibition occurs during the incubation and that the inactivation of polyphenoloxidase which is known[1] to accompany the enzymic oxidation of

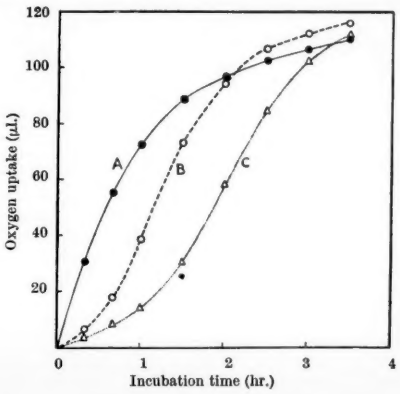

Fig. 1

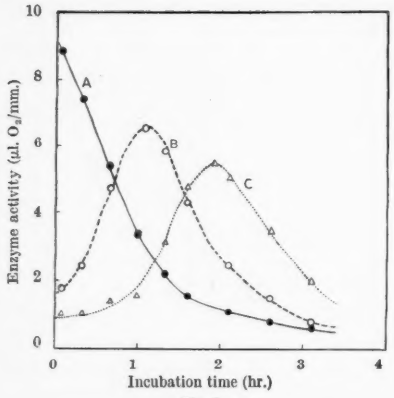

Fig. 2

phenols is delayed if thiourea is present in the medium. In consequence, a paradoxical phenomenon is observed, namely that the activity of the 'inhibited' sample after a certain time of incubation surpasses the actual activity of the uninhibited control.

A sudden disappearance of the inhibition of polyphenoloxidase by thiourea and some other compounds has already been observed by Bernheim and Bernheim[2] with the enzyme from mushroom. These authors suggested that some oxidation products of substrate may compete with the inhibitor for the enzyme surface, thus releasing the inhibition. Our experiments indicate, however, that thiourea is simply removed from the medium rather than only displaced from the enzyme surface.

The following observations appear to support this view: (1) Pre-incubation of neither the enzyme with the inhibitor nor the enzyme with the substrate is effective in diminishing the inhibition when enzyme, substrate and inhibitor are mixed together. This occurs only if these three components are incubated together. (2) The higher the remaining activity of the inhibited sample, the shorter is the period of inhibition and the earlier is the release of the inhibition. No reversal of the inhibition occurs if the concentration of thiourea is high enough (for example, 5×10^{-3} *M*) to block the activity of the enzyme completely. (3) Reducing substances, for example, ascorbate, which prevent the accumulation of oxidation products of polyphenols prolong the period of inhibition. The inhibition disappears only after all the reducing compound present has been oxidized. (4) If a fresh portion of homogenate is added to the medium at the time when the inhibition of the enzyme formerly present has been released, no inhibition of the added enzyme can be observed.

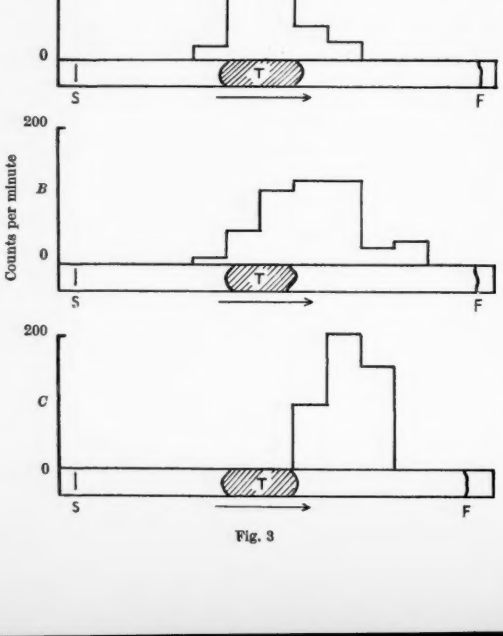

Fig. 3

A lack of a specific and sensitive analytical method for thiourea did not allow us to ascertain whether the quantity of this inhibitor diminished during the incubation. To elucidate this question experiments were performed with thiourea labelled with sulphur-35 which was prepared by thermal isomerization of ammonium thiocynate labelled with sulphur-35. The labelled thiourea was added to the incubation medium containing the homogenate and the substrate. Final concentration of thiourea was 5×10^{-4} *M*. Samples of the mixture were taken at various times of incubation. They were deproteinized by trichloroacetic acid, centrifuged, and the radioactivity was measured both in the supernatant and in the precipitate. It was observed that during the incubation the radioactive sulphur became bound to some insoluble substances insoluble in trichloracetic acid: 15 per cent of the total radioactivity was found in the trichloracetic acid precipitate after 90 min. incubation and as much as 80 per cent after 24 hr. incubation. This, however, does not explain a complete disappearance of the inhibition which was observed after 90 min. incubation. Therefore aliquots of trichloracetic acid supernatants were chromatographed together with small quantities of carrier thiourea on Whatman No. 1 filter paper in the mixture of butanol, acetic acid and water[3]. The spots of thiourea were located by spraying with ammonia-silver nitrate solution and the radioactivity was measured along the chromatogram strips. It appeared (Fig. 3) that at the beginning of incubation practically all the radioactivity was present in the spot of thiourea (*A*). After a certain time a part of the radioactivity appeared to be connected with some substances which moved faster than thiourea (*B*). When the inhibition disappeared completely, no radioactivity was found in the spot of thiourea (*C*), although about 85 per cent of sulphur-35 was still present in the supernatant.

These results indicate that during the incubation of the homogenate with phenols and thiourea, the latter, or only its sulphur atom, becomes bound to some substances which are formed during the enzymic oxidation of the substrate. These complexes seem to be no longer inhibitory against polyphenoloxidase. As sulphydryl compounds are known to combine with quinones[4], we can suppose that in our experiments thiourea, probably as sulphydryl tautometric form, combines with quinoic products of oxidation of the substrates. During a prolonged incubation these compounds presumably combine with proteins and may be precipitated by trichloracetic acid.

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Biosynthesis of some Amino-Acids from Sucrose by Germinating Uredospores of Wheat Stem Rust, Race 15B

Rust development may depend on the availability of specific nutrients, such as certain amino-acids, which are supplied by the susceptible, but not by the resistant, host plant[1]. Demonstrations of nutritional requirements by the classical deletion procedure necessitate growing the organism on a chemically defined medium. Although some plant rusts have been grown on artificial media[2], little is known about their nutritional requirements. A method permitting the determination of at least some of these requirements without the use of chemically defined diets would be useful. An indirect procedure which utilized metabolites labelled with carbon-14 when applied to the mouse[3] and blowfly[4] gave results for amino-acid requirements which agreed with the classical deletion procedure. The present report gives results of the application of this indirect method to germinating uredospores of *Puccinia graminis tritici*, race 15*B*.

Leaves and stems of the wheat plant at different stages of growth contain sucrose[5]. In addition, Shu *et al.*[6] demonstrated that uredospores of wheat stem rust, race 15*B*, during at least the initial stages of germination oxidized this sugar. Consequently uniformly labelled sucrose-^{14}C was chosen as the substrate in the present study.

About 10 mgm. of rust spores produced on Rescue wheat were evenly distributed over the surface of 5 ml. of sucrose-^{14}C solution in each of 18 Petri dishes, 100 mm. in diameter. Each dish contained about 0·05 mgm. of sucrose-^{14}C (specific activity 100 μc./mgm.) (Atomic Energy of Canada, Ltd.). The Petri dishes containing the spores were covered and held in a closed desiccator at 20° C. for 24 hr. A small container of 0·4 *M* sodium hydroxide was included in each Petri dish to absorb any carbon dioxide evolved by the germinating spores. Radioactive carbon dioxide was produced during the incubation period showing that, in these conditions and in agreement with the work of others[6], sucrose was metabolized by the germinating rust spores.

After the 24-hr. incubation period, about 75 per cent of the spores had germinated. The spores and mycelium were then collected by centrifugation, washed with water, and hydrolysed with 6 *M* hydrochloric acid for 24 hr. under reflux. The acid was removed *in vacuo* and the resulting residue was redissolved in water. The amino-acids in this solution were separated on an ion-exchange column and further purified by band paper chromatography. Final purification and subsequent quantification of the amino-acids were accomplished as described earlier[4] except that another general ninhydrin method, with slight modifications, was used[7]. Infinitely thin samples of the amino-acids plated on copper planchets were assayed for radioactivity in a windowless gas flow detector to give a probable counting error of 2 per cent. After the amino-acids were separated on paper chromatograms in *n*-butanol/acetic acid/water (4 : 1 : 5) the bands were eluted and 5 μ*M* of the authentic compound were added to each eluate. The samples, diluted with carrier amino-acid, were afterwards band chromatographed in a series of other solvents until constant specific activity was obtained. After development in each solvent the band was eluted and the specific activity of the compound was determined.

The following amino-acids were radioactive after purification to constant specific activity: α-alanine, glutamic acid, glycine, leucine, phenylalanine, proline, tyrosine, and valine. In addition, autoradiographs of chromatograms, after *n*-butanol/acetic acid/water (4 : 1 : 5) chromatography of the appropriate fractions from the ion-exchange column, showed that radioactivity was present in isoleucine, serine, and threonine. These results demonstrate that the germinating uredospores of *P. graminis tritici*, race 15*B*, can synthesize at least 11 amino-acids from sucrose. Thus, assuming that the organism continues to synthesize these amino-acids during subsequent development on the host, it seems unlikely that they are limiting factors in resistant wheat varieties[8].

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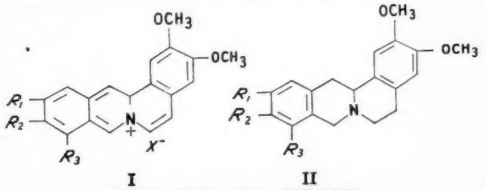
Berberine Alkaloids via 6,7-Dimethoxy*iso*quinoline-1-aldoxime

For the past two years[1], it has been clear that the development of a new and convenient method for the synthesis of the berberine alkaloids hinged on the preparation of 6,7-methylenedioxy (I)- and 6,7-dimethoxyisoquinoline aldehydes (II). The use of the first of these in the synthesis of tetrahydroberberine and related alkaloids has been reported[2]. The present communication describes the preparation and use of the dimethoxyisoquinoline aldehyde (II).



I II

III, R_1 = H, $R_2 - R_3$ = OCH_2O VII
IV, $R_1 - R_2$ = OCH_2O, R_3 = H VIII
V, R_1 = H, R_2 = R_3 = OCH_3 IX
VI, R_1 = R_3 = H, R_2 = OCH_3

Oxidation of 1-methyl-6,7-dimethoxyisoquinoline[3] afforded the 1-aldehyde (II), m.p. 176°, in 35 per cent yield (found: C, 66·18; H, 5·28; N, 6·85. $C_{12}H_{11}NO_3$ requires: C, 66·40; H, 5·07; N, 6·45 per cent). The oxime, m.p. 248° of II was quaternized with 2,3-methylenedioxy-, 3,4-methylenedioxy-, 2,3-dimethoxy-, and 3-methoxybenzyl bromides, and each of the crude salts cyclized in hydrochloric acid to afford the expected benz[*a*]acridizinium salts (III–VI) in yields of the order of 80 per cent.

The chloride of III (dihydrate) melted at 275° (*d*), while the perchlorate melted at 315–316°. The chloride of IV melted at 278–280° (*d*). The bromide of V melted at 250° (*d*), while the perchlorate melted at 312° (*d*). The 2,3,10-trimethoxybenz[*a*]acridizinium (VI) chloride (methanol-water solvate) melted at 242° (*d*), while the perchlorate (methanol solvate) melted at 310–312° (*d*).

Catalytic reduction of the first three benz(*a*)acridizinium salts (III–V) over platinum oxide yielded the expected tetrahydroberberine alkaloids. From III, tetrahydroepiberberine (± sinactine, VII) was produced in 50 per cent yield, m.p. 167–168° (refs. 4 and 5) 169–170°, 168° (found: C, 70·55; H, 6·16; N, 4·22. $C_{20}H_{21}NO_4$ requires C, 70·78; H, 6·24; N, 4·13 per cent). The base gives the reported [4,5] colour reaction in a sulphuric-acetic acid mixture. The hydrochloride decomposed at 246° (refs. 5 and 6, dec. about 286°; dec. 285–290°; found: C, 63·95; H, 5·81. $C_{20}H_{21}NO_4$·HCl requires C, 63·91; H, 5·85 per cent).

The reduction of IV yielded tetrahydropseudoepiberberine, m.p. 160 (lit.[7] m.p. 160–161; found: C, 70·85; H, 6·37; N, 4·20. $C_{20}H_{21}O_4N$ requires: C, 70·78; H, 6·24; N, 4·13 per cent). The picrate melted at 150° (d) (ref. 7. m.p. 149–150°). The reduction of V afforded ± tetrahydropalmatine (m.p. 147°) in 53 per cent yield. This material was identical in melting point, mixed melting point and infra-red absorption spectrum with an authentic sample (found: C, 71·08; H, 7·04; N, 4·24. $C_{21}H_{25}O_4N$ requires C, 70·99; H, 7·04; N, 3·94 per cent). The hydrochloride melted at 215–216° (ref. 8, 215°).

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Formation of Allantoin and Allantoic Acid from Adenine in Leaves of *Acer saccharinum* L.

Allantoin and allantoic acid have been shown to be major constituents of the xylem sap of many species of trees[1], and have been proposed as important translocatory forms of nitrogen[1,2]. Most workers in ureide metabolism in plants are of the opinion that allantoin and allantoic acid are not formed primarily by purine catabolism, as in the case in animals, but are synthesized from simpler molecules such as urea, glycine, or glyoxylate[2–4]. This communication presents evidence that allantoin and allantoic acid are readily formed from adenine in leaves of silver maple (*Acer saccharinum* L.).

Adenine-8-^{14}C was fed to two young leaves, through the petioles, in the light. After all the radioactive solution had been absorbed, the leaves were put in 1/10 strength Hoagland's solution. After 24 hr. the leaves were washed with water and extracted with 80 per cent ethanol. The ethanol extract was concentrated and chromatographed two-dimensionally in phenol/water and butanol/propionic acid/water solvents. Radioactive compounds were located by radioautography, and activities of the spots were counted directly on the paper with a Geiger–Müller tube.

The majority of the radioactivity from adenine-8-^{14}C appeared in allantoin, allantoic acid, and urea. The radioactivity expressed as percentages of total activity excluding that remaining in adenine, 26 per cent, are given in Table 1.

Table 1

Compound in leaves	Per cent radioactivity
Hypoxanthine	13
Xanthine	14
Uric acid	3
Allantoin	17
Allantoic acid	27
Urea	20
Ribosides	2
Ribotides	3
Unkonwn	1

Some of the urea may, however, have been formed from allantoic acid during the extraction. It is significant that 94 per cent of the total activity on the chromatogram was in compounds previously shown to be involved in purine catabolism in animals. The reaction sequence in maple leaves was therefore indicated to be the same as in animals, namely: adenine → hypoxanthine → xanthine → uric acid → allantoin → allantoic acid → urea plus glyoxylate. All these compounds, save glyoxylate, would be expected to acquire the carbon-14 label from adenine labelled in position 8.

The results indicate that purine catabolism is one process leading to ureide formation in higher plants. The hypothesis that ureides might also be formed directly from simpler molecules has not been investigated by me. An analysis of the xylem sap from a silver maple branch showed that allantoic acid was the major nitrogenous constituent, with glutamine second in abundance. Considerable allantoin, allantoic acid, and urea were present in the foliage. These findings indicate that allantoic acid is indeed an important translocatory and metabolic form of nitrogen in this species. Further investigations of ureide metabolism in several species of forest trees are presently under way.

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ANIMAL PHYSIOLOGY

Pathophysiological Effects of Circulating Ferritin

Ferritin is an iron-containing protein which is stored mainly in the spleen, liver and marrow. The richest source of ferritin is the reticulo-endothelial system of horse spleen ; but there is no ferritin in the blood of a healthy horse.

In previous studies[1,2], a large quantity of ferritin was proved, by the precipitin test and the complement fixation test[1] to be circulating in the blood of horses suffering from infectious anæmia.

On the other hand, several workers have obtained results which suggest that a small quantity of ferritin (nitrogen 0·0005 μgm./0·5 ml.) has an effect on the vasodepressor mechanism[3,4] which inhibits the constrictor response of the muscle capillaries in the

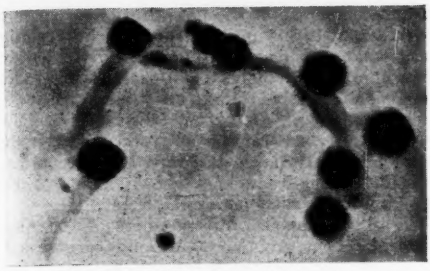

Fig. 1. Crystals of the circulating ferritin from the serum of a horse suffering from infectious anæmia

mesoappendix of the rat to topical adrenaline, and that this effect plays an important part in the regulation of the peripheral circulation. They further suggest that this vasodepressor activity of ferritin is due to the presence of the surface iron[5,6].

In this work, the relationship between the circulating ferritin and the clinical symptoms in infectious anæmia in horses has been made clear. Also, the pathophysiological changes in horses, goats, rabbits and other animals, after intravenous injection of a large quantity of crystalline horse spleen ferritin, purified by the method of Mazur and Shorr[3] have been investigated.

It was found that the intensity of febrile attack, leucopænia and the functional obstacles in circulation, respiration and exercise, etc. in infectious anæmia of horses, was closely related to the amount of ferritin (nitrogen 0·1–10 μgm./ml. serum) in blood from the jugular veins. In particular, the extraordinarily high ferritin nitrogen concentrations of 5–10 μgm./ml. were found, by means of the precipitin test and the complement fixation test, in the sera of horses which fell after presenting serious symptoms. Serum taken from a horse during a febrile attack was centrifuged at 40,000 r.p.m. (105,500 *g*) for one hour and ferritin crystals were observed after the addition of cadmium sulphate (Fig. 1).

In other experiments considerable amounts of the crystalline horse spleen ferritin, containing 50–100 mgm. of nitrogen, were injected intravenously into healthy horses with an average weight of about 250 kgm. (nitrogen 0·2–0·4 mgm./kgm. body weight) the concentration of ferritin in sera from the jugular veins reaching 5–10 μgm. nitrogen per ml. After this treatment the horses began to show the signs of lassitude and weakness, especially in the hind legs, 20–50 minutes from the time of the injection, and they fell about 60 minutes after the injection, showing acute symptoms similar to those of infectious anæmia in horses. At the same time, the horses showed such striking pathophysiological changes as a rise of the pulse rate, the small and weak heart-beats, a fall of blood pressure, congestion of the jugular vein, obstacles to peripheral circulation, increase in respiration, dyspnea and leucopænia, etc. Upon recovery, after 30 to 90 minutes, the horses rose and showed signs of shivering, especially in the hind legs, followed by the febrile attack.

Next, similar experiments on the pathophysiological effects of crystalline horse spleen ferritin were carried out in goats with average weights of 10–15 kgm. The goats showed obvious pathophysiological changes similar to those induced in horses. Intravenous injections of horse spleen ferritin containing 5–25 mgm. of nitrogen (0·4–2 mgm./kgm. body weight). The goats fell after about 60 minutes, and the febrile attack occurred after about 3 hours.

Rabbits, however, with average weights of 2·5 kgm. showed anæmia, leucopænia and the febrile attack after an intravenous injection of horse spleen ferritin (1–5 mgm. nitrogen ; 0·4–2 mgm./kgm. body weight), but showed scarcely any other obvious changes. Dogs, guinea pigs and mice also showed leucopænia and the febrile attack, but exhibited a strong resistance to the toxicity of horse spleen ferritin.

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Post-activation Changes at the Mammalian Neuromuscular Junction

An attractive explanation of the post-activation potentiation found at the neuromuscular junction is that the presynaptic impulse, in addition to producing a momentary synchronous release of miniature potentials—the endplate potential—causes mobilization of further transmitter signalled by an increase in the frequency of the spontaneous miniature discharge[1]. This hypothesis, if confirmed, would be of great importance in understanding the mechanism of transmitter release. It has therefore been investigated by studying changes in the frequencies of the miniature potential and in the size of the parallel endplate potential after conditioning by one to five thousand volleys, using a wide range of stimulating frequencies.

Intracellular recording (glass electrodes filled with 3 *M* potassium chloride) from the isolated rat diaphragm, bathed in oxygenated Krebs solution, was used in all experiments. In most experiments miniature potentials and endplate potentials were studied in the same fibre, 0·012 *M* magnesium chloride being added to the bath. In some experiments endplate potentials alone were studied using curare (1×10^{-5} *M*). Stimulation via the phrenic nerve was always supramaximal. The temperature was maintained at 33–37°C.

For small numbers of conditioning volleys (1–100), changes of miniature potential frequency were followed by pooling large numbers (more than 80) of serial observations on the same fibre, to obtain statistically significant results. The corresponding endplate potential changes were examined in curarized muscle. With large numbers of stimuli, a comparison of the frequency of the post-tetanic miniature potentials or size of endplate potential with the pretetanic controls in the same experiment was usually significant.

As previously reported[2], it was found that one impulse was followed by an acceleration of frequency of miniature potential lasting 200 msec. This effect was maximal within the first 10 msec. after the conditioning volley and declined very rapidly afterwards (Fig. 1*a*). Within this same 10 msec. interval in the curarized preparation, a testing impulse elicited an endplate potential which was often larger than the control. As described previously[2] (using extracellular recording), increasing depression of the size of the testing endplate potential was found for 200–300 msec. after a conditioning volley. The time course of this increasing depression paralleled the

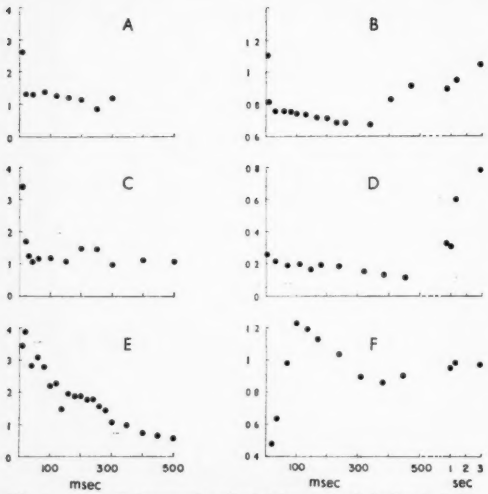

Fig. 1. *A*, *C*, *E*, frequencies of miniature potential after 1, 5, 20 conditioning volleys at 200/sec. (10 m*M* magnesium chloride). *B*, *D*, *F*, endplate potentials after the same number of volleys (curarized preparation).
Ordinates. Ratio of post-activation frequency (or size of endplate potential) to control frequency (or size of endplate potential).
Abscissæ. Same time-scale for all graphs. Note break in scale in *B*, *D*, *F*.
In *A*, *C*, *E*, each dot represents the average frequency over the period between it and the preceding dot. In *B*, *D*, *E*, each dot represents the average of three endplate potentials, elicited by testing volleys at stated interval after tetanus. 10 sec. was allowed between each combination of tetanus and testing volley.

decline of the potentiation of the frequency of miniature discharge (Fig. 1*A*, 1*B*), supporting the interpretation of this increasing depression as the resultant of a potentiation of the endplate potential submerged by a larger depression.

Increasing the number of conditioning impulses (1–5 at 200/sec.) produced further depression of the size of the testing endplate potential, the phase of increasing depression immediately after the tetanus being retained. The frequency change of miniature potentials, however, was always an acceleration, subsiding over the period of increasing depression of the endplate potential (Fig. 1*C*, 1*D*).

Ten to fifty conditioning stimuli (at 200/sec.) increased the duration and size of the acceleration of the miniature potentials. If the corresponding period of time was explored in the curarized preparation, a type of early potentiation of the endplate response could be demonstrated, analogous to that found in the spinal cord[4] (Fig. 1 *E*, *F*).

Larger numbers (100–4,000) of conditioning stimuli were explored over a wide range of frequencies (0·2–400 sec.) and durations (0·4–10 sec.). It was confirmed[5] that, in magnesium poisoned muscle, the post-activation potentiation of the frequency of miniature discharge of the endplate potential run a similar time course. However, while the maximum discharge frequency was attained immediately after the conditioning volley, the endplate potential size was usually not maximal for 2–3 sec. (Fig. 2).

The frequency of miniature potentials was always increased following activity. The duration of the effect depended on both the numbers and frequency of the conditioning volleys. The maximum potentiation of the miniature discharge also varied with the frequency of stimulation, but an interesting discontinuity in this relationship was found. There appeared to be a critical frequency (100/sec.) at or above which increasing numbers of volleys caused a steep linear increase in the potentiation of frequency of discharge, up to the limits of the counting technique. Lesser frequencies, no matter how many volleys given, (for example, 2,000 volleys at 50/sec.) never produced an increase greater than 5–6 times the resting frequency (per sec.). This may be related to the fact that the increased release of miniature potentials after an impulse is large for only 10 msec. and thus only stimulating frequencies of 100/sec. or more would have a marked summing effect.

After all frequencies and numbers of stimulating volleys tried, the frequency of miniature potentials was always increased—depression was never found. Thus if the frequency of miniature potentials is an index of mobilization of transmitter, an impulse has a uniformly facilitatory effect upon the succeeding endplate potential. Further, the phase of submerged potentiation after a few impulses, of early potentiation and of late potentiation, all occur during periods when the frequency of the miniature potentials is enhanced.

It must be admitted that there is a decoupling between the availability of transmitter as measured by frequency of miniature discharge, and the amount of transmitter actually released by a nerve impulse in certain circumstances, for example, the effect of high magnesium concentrations in markedly reducing or abolishing the endplate potential without affecting

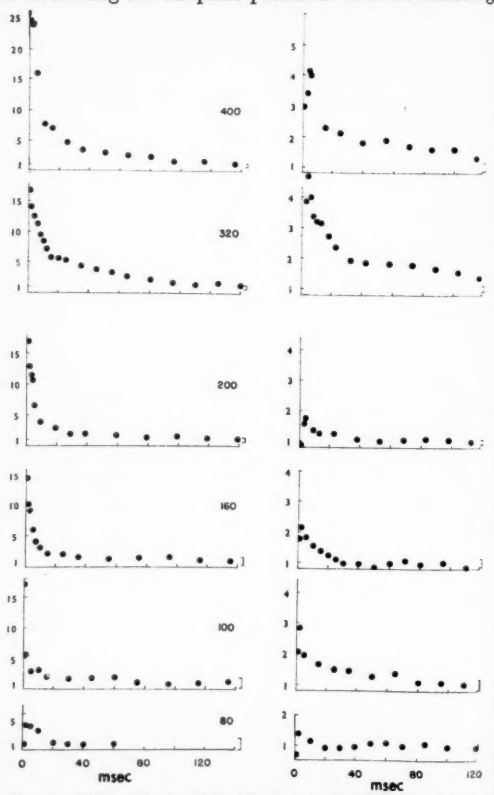

Fig. 2. Effect of tetanus for 7 sec. at stated frequency on frequency of miniature potential and size of endplate potential size, recorded simultaneously in the same fibre. 12 m*M* magnesium chloride, 37°C.
Ordinate. Ratio post-activation frequency (or endplate size) to control.
Abscissæ. Time-scale common to all graphs.
Each dot represents average of frequency of miniature potential (or size of endplate) over period between it and its predecessor.
Brackets at end of abscissæ mark ±2 standard deviations of pretetanic frequency (or size of endplate potential)

the frequency of miniature potentials, and the delayed potentiation of the endplate potential after a tetanus in high magnesium solutions. Other factors, notably depletion of available transmitter, are obviously also concerned in determining the amount of transmitter released. Mobilization of transmitter by preceding impulses remains an important, indeed probably the basic factor, in post-activation potentiation.

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Oxygen Uptake of Glycerol-extracted Muscle Fibres

IN the ten years that have passed since Szent-Györgyi[1] introduced the use of the glycerol-extracted muscle fibre, it has proved extremely useful for investigating the molecular events underlying muscular contraction. Usually, the fibre has been regarded as a 'contractile skeleton'; on this basis its response to a chemical agent has commonly been interpreted as a direct response of the contractile elements, rather than an effect mediated or influenced by other still functional components of the muscle cell.

When exploratory studies of the activities of adenosine triphosphatase (Wilson, J. A., and Guthe, K. F., unpublished work) suggested that glycerol fibres take up inorganic phosphate under certain conditions, the question arose as to the possible persistence of a broad range of enzymes and enzyme systems in the fibre. Although Perry[2] has directed attention to the desirability of investigating this matter, little information is at present available on the properties of the fibre apart from the contractile system itself and one or more relaxing factors[3]. As a preliminary approach to the question, we have sought in the present study to determine the respiratory capacities of glycerol-extracted rabbit psoas fibres.

Freshly removed fibres were stored in 50 per cent glycerol (v/v) at −17°C. for periods of four weeks to four months. As desired, they were removed from the glycerol, rinsed briefly in 0·25 *M* sucrose, and homogenized in 9–30 volumes of fresh 0·25 *M* sucrose. Manometric measurements of the oxygen consumption of such homogenates are illustrated in Fig. 1. Curve A_1 shows the endogenous oxygen uptake of a homogenate of fresh fibres prior to storage in glycerol, and curve A_2 the slightly lower rate exhibited by the same homogenate in the presence of 0·0017 *M* succinate. In curves *B* and *C* are illustrated the oxygen consumptions of other homogenates, prepared from fibres stored in glycerol for five weeks and four months, respectively. Of particular interest is the fact that the homogenates of glycerol fibres consume oxygen at rates substantially similar to those shown by homogenates of fresh fibres.

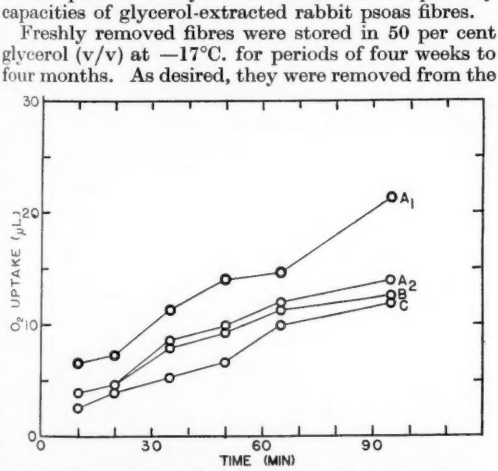

Fig. 1. Oxygen consumption of rabbit psoas fibre homogenates at 37°C. In all experiments, a total flask volume of 1·8 ml. was used, containing 0·045 gm. tissue. Final concentrations were as follows: sucrose, 0·21 *M*, and phosphate, 0·017 *M* (*p*H 7·0). Curve A_1, fresh fibre without succinate; curve A_2, fresh fibre in 0·0017 *M* succinate; curve *B*, 5-weeks glycerol-extracted fibre in 0·0017 *M* succinate; curve *C*, 4-months glycerol-extracted fibre in 0·0017 *M* succinate

These results have been confirmed by additional experiments, which have shown also that homogenates of fibres stored six weeks in glycerol consume oxygen as rapidly without added substrate with succinate.

The above findings imply that a functional respiratory enzyme system is retained in the glycerol fibres. Support for this view was obtained from spectroscopic observations, which revealed that an intact cytochrome system persists in the glycerol fibres even after four months. Although the glycerol fibres, like fresh fibres, contain relatively low concentrations of cytochromes, the absorption bands of components $a+a_3$, *b*, and *c* were in all cases readily detectable in the presence of sodium dithionite, and particularly after intensification of the absorption spectra at the temperature of liquid nitrogen. The relative band intensities in fresh fibres were observed to be $b=c>a+a_3$; those in the glycerol fibres $b>c>a+a_3$, suggesting that a small part of the cytochrome *c* may be extracted from the fibres during the period of storage at low temperature.

Further evidence for the integrity of the cytochrome system after storage in glycerol was provided by comparative studies on rabbit liver mitochondria, isolated in 0·25 *M* sucrose. The mitochondria, after storage for six weeks in 50 per cent glycerol at −17°C., consumed oxygen at a rate about ten times that of the muscle homogenates. This oxygen uptake was almost entirely abolished in 0·001 *M* cyanide.

These findings indicate that the glycerol-extracted muscle fibre, as customarily prepared for physiological studies, contains respiratory substrate and the means for oxidizing it. It is perhaps not surprising that the fibre respires, in view of the marked vitality of many types of animal cells after low-temperature storage in glycerol media. Clearly, a fibre that not only contracts but also respires may well retain other significant biochemical activities. The latter unquestionably merit attention in assessing the contractile properties of the fibre. In particular, the status of the mitochondrial oxidative phosphorylation mechanism becomes an important point; if it remains intact, the products of phosphorylation and transphosphorylation could themselves exert potent effects on the contractile mechanism, as well as influence its response to a wide range of physiologically active substances.

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Effect of Ethylenediamine Tetra-acetate on Vitamin B_{12} Absorption in the Rat

Herbert[1] has reported that calcium is obligatory for absorption of vitamin B_{12} by liver slices in the presence of intrinsic factor, since the addition of a calcium binder—the di-sodium salt of ethylene diamine tetra acetate (EDTA) decreased the intrinsic factor effect. Minard and Wagner[2] made similar observations with liver homogenates in which calcium increased vitamin B_{12} uptake in the presence of intrinsic factor.

The *in vivo* work on this problem has not been so direct, nor is there agreement concerning the requirement for calcium for vitamin B_{12} absorption from the gut of the living animal. Abels *et al.*[3], in studies with gastrectomized rats, administered vitamin B_{12} — ^{58}Co with neutralized rat gastric juice, and observed no impairment of intestinal absorption of vitamin B_{12} when large amounts of ethylene diamine tetra acetate were added to the administered materials. Gräsbeck and Nyberg[4] administered ethylene diamine tetra acetate to normal human subjects along with a test dose of radiovitamin B_{12} and showed a definite decrease in vitamin B_{12} absorption which was reversed by simultaneous administration of calcium lactate. Gräsbeck *et al.*[5], have also implicated calcium in the failure of steatorrhea patients to absorb vitamin B_{12}.

In the course of studies on agents which would promote vitamin B_{12} absorption, both the di- and tetra-sodium salts of ethylene diamine tetra acetate were tested for their effects on vitamin B_{12} absorption in the rat. Intact rats received daily 1 μgm. of vitamin B_{12} — ^{60}Co in 2·5 ml. of solution for three consecutive days. The test vitamin doses were contained in water, 2 per cent or 4 per cent solutions of di-sodium ethylene diamine tetra acetate, or 2 per cent or 4 per cent solutions of tetra-sodium ethylene diamine tetra acetate. Forty-eight hours after the final test dose the animals were killed and the radioactivity levels in their blood, livers, and kidneys were estimated by scintillation counting. The results for the kidneys as per cent of the radioactivity administered as vitamin B_{12} — ^{60}Co per gram of tissue are presented in Table 1. Liver and blood values showed differences of the same order between the control and experimental groups in all cases and are therefore not given.

Table 1. Per Cent Vitamin B_{12}-^{60}Co Found in Kidneys 48 Hours After Final Oral Dose

	Administered with vitamin B_{12}-^{60}Co					
	Tetra-sodium EDTA			Di-sodium EDTA		
	0 per cent	2 per cent	4 per cent	0 per cent	2 per cent	4 per cent
Per cent of radioactive dose per gm. of kidney	1·50 3·07 2·02 1·62	1·92 1·58 1·30 1·43	1·84 1·64 3·32 1·76	1·40 1·34 1·90 2·08	2·98 2·97 1·66 2·05	2·70 2·72 4·80 3·13
Average ± S.E. of Mean	2·05± 0·357	1·56± 0·133	2·14± 0·396	1·68± 0·183	2·42± 0·333	3·34± 0·497
p Value* vs. H_2O	—	0·15	0·5	——	0·1	0·05

* One-sided t test.

Administration of the test dose of vitamin B_{12} in either 2 per cent or 4 per cent solutions of tetra-sodium ethylene diamine tetra acetate caused no observable change in the tissue vitamin B_{12} — ^{60}Co levels over that observed with the water solution. When the test dose was administered in di-sodium ethylene diamine tetra acetate, both 2 per cent and 4 per cent solutions caused an increase in the tissue radioactivity as compared with that of the control (water) group.

In no case was there a significant decrease in vitamin B_{12} absorption due to concomitant administration of vitamin B_{12} and ethylene diamine tetra acetate. A decrease had, however, been expected in the case of tetra-sodium ethylene diamine tetra acetate because the pH of this salt in solution (approximately pH 11) is unfavourable to the stability of the vitamin. The increase in vitamin absorption found with the di-sodium ethylene diamine tetra acetate (pH 5) may, on the other hand, be the result of a pH buffering or of a weak chelating effect which stabilizes the vitamin in the gut, and not due to the removal of some interfering substance by chelation with the ethylene diamine tetra acetate. Unfortunately, it is not clear which salt of ethylene diamine tetra acetate was used by Abels *et al.*[3] or by Gräsbeck and Nyberg[4], although Abels *et al.*, did point out that Herbert[1] had used the di-sodium salt in his *in vitro* experiments.

There is no evidence of a calcium requirement for vitamin B_{12} absorption under the conditions of our experiment; indeed, it was noted that as the amount of di-sodium ethylene diamine tetra acetate administered was increased vitamin B_{12} — ^{60}Co absorption was increased. The relatively large amount of the vitamin used in our experiments makes it possible that some secondary mechanism might be operative in the absorption process. These results in intact rats substantiate the findings of Abels *et al.*[3], in gastrectomized animals.

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Heat Inactivation of Rinderpest-infected Bovine Tissues

Information on the survival of rinderpest virus in stored infected bovine tissues is pertinent to the problem of the export of meat from rinderpest-endemic areas. The literature contains numerous snippets of contradictory information[1]. The confusion has arisen through the use of thermal death points which are meaningless and fail to express the response of a virus to heat. Accordingly, a fresh appraisal of the effect of heat on the infectivity of rinderpest-infected bovine tissues was made.

The experimental design was factorial such that the heat stabilities of three strains of rinderpest virus in the form of three types of infected bovine tissues were determined at four temperature-levels over the range 7–56°C. In addition, immediate and storage death at temperatures below the freezing-point were examined. The rinderpest strains were the virulent Kabete 'O', the attenuated Kabete caprinized and the attenuated Nakamura III lapinized. The two latter are widely used in equatorial Africa as live virus vaccines. The 'O' and the caprinized strains were titrated in cattle and the lapinized strain in rabbits.

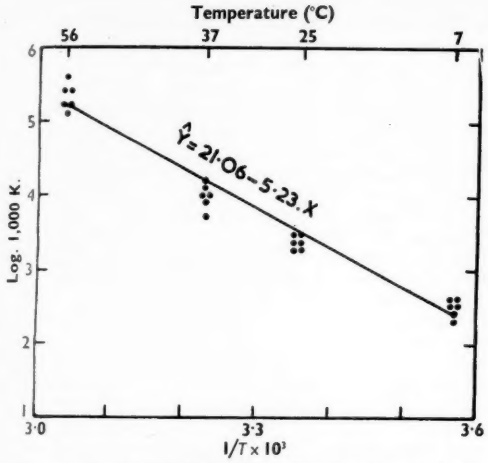

Fig. 1. Arrhenius plot of heat inactivation of rinderpest-infected bovine lymph glands and spleens

The bovine tissues used were the carcase lymph glands, spleen and blood. They were selected because of their known *p*H stability[2]. Groups of three donor cattle inoculated subcutaneously with 1,000 CID_{50} of either the 'O', caprinized or lapinized strains were bled and killed when virus growth had reached its peak. Tissues were harvested and divided into cubes. Each storage aliquot consisted of two tissue cubes, each from different animals. The aliquots were stored in screw-capped bottles and were distributed at random at 56, 37, 25, 7 and −15°C. Titres of zero-hour aliquots were estimated immediately in suitable indicator hosts. At intervals, further aliquots were selected at random and their virus content estimated. All experiments were replicated.

Heat inactivation of rinderpest virus in infected lymph glands and spleens occurred at all temperatures tested and proceeded as a reaction of the first-order (Fig. 1). The response of the infected tissues was homogeneous and independent of the strain of the virus. The activation energy of heat inactivation was 24 kilocalories per mole and the entropy of activation was +82 calories per degree per mole. Inactivation of rinderpest-infected bloods also occurred at all temperatures tested but the inactivation rates were significantly slower than in other tissues at 25° and 37°C.

The half-life periods of infected lymph glands, spleens and bloods were 5 min. and 2·3 days at 56 and 7°C. respectively. The half-life periods of infected lymph glands and spleens at 37 and 25°C. were respectively 105 min. and 6·4 hr. whereas the half-life periods of infected bloods were significantly longer, being 21 hr. at 37°C. and 1·5 days at 25°C.

Extrapolation of individual Arrhenius plots to temperatures below the freezing-point gave values which were significantly lower than the actual observed values. For example, the expected half-life at −15°C. in tissues other than blood was 120 days whereas the mean observed half-life was only 72 days. The discrepancy was believed to be a function of the freezing (and thawing) process because of the significant difference in the mean titres of supercooled and frozen infected bloods held for the same time at the same temperature.

The carcase from an animal which is clinically sick will not pass inspection because of its fevered state. Similarly the chances of a carcase from a convalescent but still infectious animal being passed for human consumption are slim because of the characteristic dehydration and emaciation associated with rinderpest. Unfortunately, however, the eclipse phase of rinderpest virus multiplication is shorter than the incubation period of the disease and appreciable titres of virus can be found in clinically-normal cattle. Such animals, if slaughtered, would pass inspection and, if exported, would carry viable virus. Our findings suggest that normal meat trade methods designed to conserve beef will preserve rinderpest virus. Consequently the veterinary authorities of countries where rinderpest is endemic or where vaccination with live attenuated virus is practised can never guarantee that carcases exported from these areas are free of rinderpest virus. Trade remains impossible if the criterion of safety is a warranted virus-free product.

Details of these experiments will be published elsewhere. Financial support from the Kenya Government and the Foreign Operations Administration of the United States of America is gratefully acknowledged.

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Absorption of Atmospheric Moisture by Freeze-dried Pork and Fish

Recent work at this Establishment has re-emphasized the need for low moisture content in dehydrated meat and fish if these are to have a long storage life[1]. However, moisture determinations on foodstuffs dried to a low moisture-level by the accelerated freeze-drying process[2] gave erratic results. The method used was to grind the material to a powder in a household mincer; portions of this powder were weighed into moisture tins which were then held for 5 hr. in a vacuum oven at a temperature of 70°C. (158°F.) and a pressure of less than 3 mm. mercury. During this period, a very slow stream of air dried over sulphuric acid was allowed to leak into the oven.

The above method was modified so that the exposure of the foodstuff to air was reduced to a minimum by keeping material in air-tight jars from the moment of preparation until analysis, and grinding it directly into weighed moisture tins. The results became more reproducible and the duplicates better, which indicated that the absorption of atmospheric moisture was the cause of the previous difficulties. To confirm this, the rate of uptake of moisture of dehydrated pork and dehydrated cod was followed by weighing, at intervals, samples exposed in open dishes. These samples consisted of pieces of roughly 2 cm. cube or of powder spread in a layer about 0·5 cm. deep. The initial and final moisture contents were also determined; the initial moisture content plus the gain in weight gave a theoretical final moisture content which could be compared with that determined, so checking the accuracy of the methods. In each case the calculated moisture content after 24 hr. agreed to within 0·1 gm./100 gm. solids or non-fatty solids with that determined. The results of this test are shown in Fig. 1.

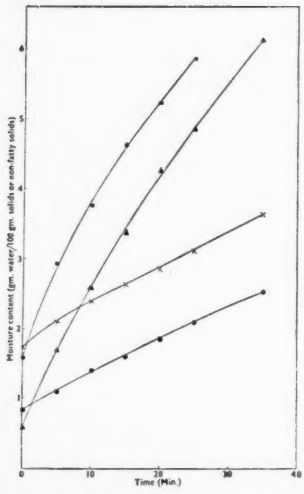

Fig. 1. Uptake of atmospheric moisture by dehydrated pork and dehydrated cod. ●, pork, pieces ; ▲, pork, powder ; ×, cod, pieces ; ■, cod, powder.
The temperature varied from 20° to 23°C. and the relative humidity from 42 to 50 per cent. The moisture contents are expressed as gm. water per 100 gm. non-fatty solids for pork and per 100 gm. solids for cod

It is clear that the uptake of moisture by both pieces and powder is very rapid. Exposure of the sample to the atmosphere, especially after grinding, would have a very marked effect on the accuracy and reproducibility of moisture determinations. Unless particular attention is given to this point, moisture determinations in well-dried foodstuffs will give figures appreciably higher than the true values.

These results have applications in a wider field than laboratory moisture determinations. They emphasize the known desirability of handling freeze-dried materials such as proteins, enzymes, sera and bacterial cultures in such a way that exposure to moist air is a minimum. They are also relevant to the commercial dehydration of food to low moisture contents. The difference between the storage life of dehydrated meat or fish at 2 per cent and at 4 per cent moisture is several months at 37°C. (100°F.). Under temperate conditions, the difference is likely to be measured in years, and it is therefore of considerable importance that the dried foodstuff should not be allowed to pick up appreciable moisture.

I wish to thank Miss Patricia Greenway for technical assistance in this work.

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Noradrenaline and Adrenaline in the Rat Brain during Acute and Chronic Morphine Administration and During Withdrawal

Following the demonstration of a release of catecholamines by morphine from the brain stem of cats and dogs[1], my interest became focused on the mechanism of this release and the possible role of brain catecholamines in the morphine abstinence syndrome. In many this syndrome is characterized by signs of autonomic activity, for example, tremor, pilo-erection, lachrymation, salivation, fever, excitement mydriasis[2]. The same symptoms can be induced experimentally by electrical stimulation of hypothalamic and limbic brain areas of various laboratory animals[3] and chemically by interference with the brain-stem content of some biogenic amines[4,5].

Two series of experiments will be reported here.

In the acute experiments male and female rats weighing 150–250 gm. were given morphine hydrochloride intraperitoneally in a dose of 20, 30, 60 and 90 mgm./kgm. body-weight at 9 a.m. In the chronic experiments morphine was given at 9 a.m. and at 5 p.m., the dose-level being increased from 20 to 200 mgm./kgm. body-weight within three weeks.

The animals were killed by decapitation, the whole brains were immediately dissected out and extracted in 10 per cent trichloracetic acid. Five brains were pooled. For the purification of the extract a modification of the method of Euler and Orwén[6] was used. In order to avoid adsorption of substances interfering with the biological assay, it was found necessary to reduce the amount of aluminium oxide to 0·5 gm. per each five rat brains. The final estimation of the catecholamine mixture was made by the Euler's method[7] on the cat's blood pressure and the hen's rectal cæcum.

The results of the acute experiments are presented in Table 1. One single injection of 0·5 ml. of physiological

Table 1. Content of Noradrenaline and Adrenaline in Rat Brains During Acute Experiment—One Injection at 9 a.m. The Animals Killed 2–8 hr. after Injection

No. of rats (deg. of freedom)	Treatment and dose	Noradrenaline mgm./gm. wet weight mean ± *S.E.*	Adrenaline	Per cent adr./nor. + adr.
55 (11)	Controls	217 ± 15	9·8 ± 1·3	4·3
75 (15)	Morphine 30 mgm./kgm.	91 ± 7·3	9·9 ± 1·5	9·8
40 (8)	Morphine 60–90 mgm./kgm.	310 ± 32	10·9 ± 2·7	3·4

1 ngm. = 10^{-9} gm.

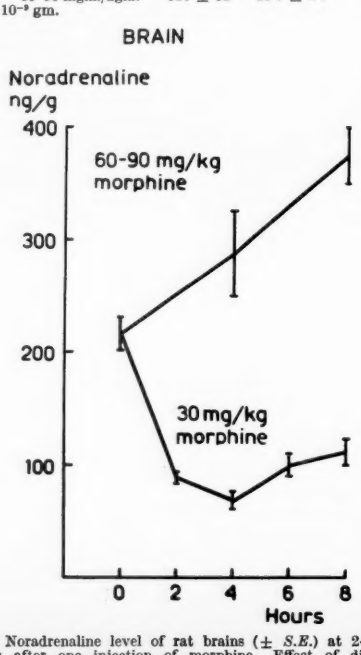

Fig. 1. Noradrenaline level of rat brains (± *S.E.*) at 2-hourly intervals after one injection of morphine. Effect of different dose levels

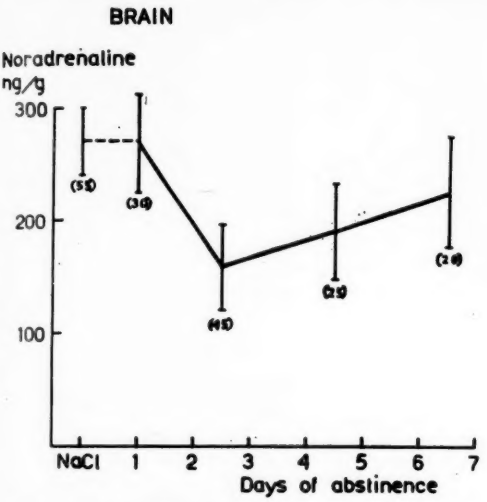

Fig. 2. Noradrenaline level of rat brains (± *S.E.*) during abstinence period following three weeks of morphine treatment, 20–200 mgm./kgm. For comparison the level of the saline-treated controls is included as a starting level. Number of rats within brackets

saline seemed to have no effect on the catecholamine content of the brain, and no indication of diurnal variation was obtained. Animals treated with saline as well as untreated animals could therefore be brought together as a control group. Administration of a single dose of 20 mgm./kgm. morphine failed to bring about any change in the noradrenaline or the adrenaline content of the brain. A single dose of 30 mgm./kgm. resulted 2–8 hr. after administration in a highly significant reduction of the noradrenaline content by 58 per cent ($P < 0.001$). No concomitant change of the adrenaline-level was observed, the relative amount of adrenaline during this period hence increasing from 4·3 to 9·8 per cent. In Fig. 1 the lower curve shows the mean noradrenaline values (± *S.E.*) at 2-hourly intervals. After 24 hr. the level was back within the normal range.

When the morphine dose was increased to 60 and even 90 mgm./kgm., contrary to expectation, the noradrenaline reduction failed to appear, the values found actually showing an increase of 43 per cent ($P < 0.02$). No change in the adrenaline values was seen. This finding might support the view that the catecholamine release during acute experiments is, at least partly, an effect of hypoxia[8]. Vogt[1] reported a release of catecholamine in cat brains following severe anoxia. The depressant respiratory effect of small morphine doses tends to disappear at higher dose-levels, near to the convulsion threshold, where respiration may even be stimulated[9,10].

Fig. 2 illustrates the brain-levels of noradrenaline during the long-term experiments. Three weeks of saline treatment caused a slight increase of the mean values as compared with the controls of Table 1. At the same time the values increased. The morphine-treated animals killed 24 hr. after the last injection (1 day's abstinence) had a mean value very close to these saline-treated controls. The two values have been connected in the figure by a broken line. The noradrenaline-levels of the remaining abstinence days have been combined, each point representing the level of two consecutive days (mean ± *S.E.*). On the second and third abstinence days there was a drop of the noradrenaline level followed by a return to the normal level within the next few days. The difference between the saline-treated controls and the value of the second and third abstinence days is significant ($P < 0.05$).

The low noradrenaline figures were not accompanied by any significant change of the adrenaline-level. The mean adrenaline value for the saline-treated controls was 9·6 ± 2·0 and for the morphine-treated rats during the entire experiment (1st to 7th abstinence day) 11·3 ± 1·4 ngm./gm. (1 ngm. = 10^{-9} gm.).

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Visual Spatial and Temporal Summation

THE interpretation of the laws of spatial and temporal summation, found to be valid at the threshold of peripheral vision, is of importance for the understanding of the mechanism of retinal excitation. Van der Velden[1] was the first to give a rational interpretation of this problem. He showed that if the absorption of two light quanta elicits a threshold sensation of light, the only laws of spatial and temporal summation compatible with such a mechanism are those which are actually observed. Square root laws, namely Piper's Law for spatial and Pieron's Law for temporal summation, are of the form:

$$X^{\frac{1}{2}} \times I = \text{constant} \qquad (1)$$

where X represents the area or the exposure time of the test, and I the threshold stimulus.

The square root law for areal variation is observed rigorously when the stimulation time $t < t_0$ and the diameter of the stimulus area $d > d_0$; the corresponding temporal law applies when $d < d_0$ and $t > t_0$. d_0 and t_0 are experimental constants; t_0 is 0·1 sec. between eccentricities of 11 and 19° (Baumgardt and Hillmann[2]) and d_0 is equal to 45 min. of arc[3] in the same retinal region. These constants indicate the value of the parameter time or diameter—below which integration is complete, that is to say the Bunsen–Roscoe and Ricco's Laws are applicable. Beyond these values there is an intermediate range which leads to the values for which the square root laws are applicable. As regards spatial summation the intermediate range lies between 45′ and 100′ approximately; it is rather difficult to determine the intermediate range for the temporal parameter.

Baumgardt[3] finds that Pieron's Law applies within a range of between 0·1 and 3 sec. Bouman and van der Velden[4] verified Piper's Law with test fields as large as 23·2° in diameter.

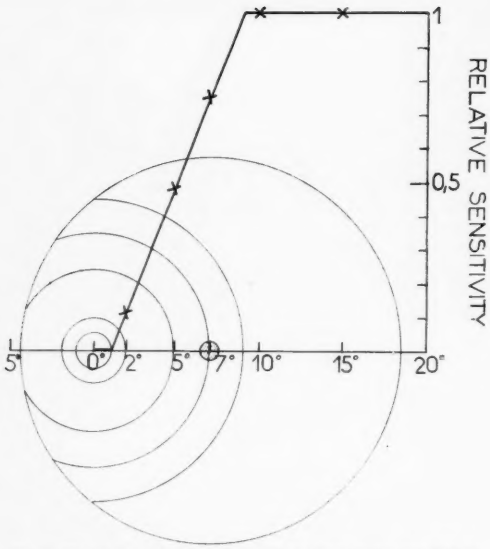

Fig. 1. Shaded circle, test field of 1° (ref. 7); large circles, test field of 23·2° (ref. 4); ordinate (×), relative sensitivities (ref. 7); circular areas, curves of equal sensitivity; 0° represents the foveal centre

According to van der Velden, the number n of quanta absorbed at the visual threshold in a domain d, t such that $d \leqslant d_0$ and $t \leqslant t_0$ must necessarily be equal to 2. But Baumgardt[5] has shown that the square root laws apply equally when n is equal to $2u$, where $u = 2, 3, 4. \ldots$. When $d \leqslant d_0$ at the same time as $t \leqslant t_0$ the second quantum of each pair of quanta absorbed can simultaneously represent the first quantum of the following pair. Consequently, n can be odd or even.

Numerous researches published since 1942 have shown that n is certainly larger than 2 and mostly lies between 5 and 8. Consequently, van der Velden's hypothesis has to be abandoned.

A hypothesis of Barlow's[6] postulates the existence of 'dark noise', biological in character, the intensity of which sets a limit to the visual threshold. As this hypothesis is likewise tied to the existence of square root laws, it is particularly important to examine their validity.

Pirenne has questioned the validity of Piper's Law on numerous occasions. Pirenne and Marriott[7] have presented the most cogent arguments. They criticize the manner in which Bouman and van der Velden have verified Piper's Law, these authors having worked with test fields centred at a point 7° outside the fovea. Having measured the threshold in various parts of the region studied by Bouman and van der Velden, Pirenne and Marriott stated that it shows a large variation with retinal position. They conclude that if the work of these authors is sound, their results prove that Piper's Law is observed as a correct area threshold relation only when such a concept is applied to a retinal region of variable sensitivity. It is on this criticism that they base their rejection of the validity of Piper's Law. The argument appears to be a feeble one especially as the validity of Piper's Law has been verified in valid circumstances by other workers, for example, by Baumgardt[3], between 11 and 19° from the fovea. This is a region in which even Pirenne and Marriott have observed the sensitivity to be constant. These authors omit to support their criticism by numerical argument and are content to assert that the result is wrong because it is based on the false idea of a constant retinal sensitivity. In order to test this conclusion, one can utilize the relative sensitivity values of Pirenne and Marriott for a 1° field applied at various points between 0 and 20° from the fovea. For a constant stimulus the probability of absorption of a quantum within any one of a given zone below the graph is proportional to its area and sensitivity. Calculation shows that under these conditions the effective absorption in a retinal area of 23·2° in diameter centred at an eccentricity of 7° is 584 times higher than the absorption within a concentric area of 1° in diameter. Defining the latter as unity it is found that if Piper's Law is applicable:

$$I = 1/\sqrt{584} \text{ or } \log_{10} I = 2{\cdot}616 \qquad (2)$$

If one neglects the non-uniformity of retinal sensitivity as was done by Bouman and van der Velden, the application of Piper's Law yields:

$$I = 1/\sqrt{(23{\cdot}2)^2} = 1/23{\cdot}2 \text{ or } \log_{10} I = \bar{2}{\cdot}634 \qquad (3)$$

The logarithm of the ratio of the values calculated from (2) and (3) is equal to 0·018. The standard error of these measurements cannot be less than 0·05; hence, the difference is scarcely significant.

In conclusion, it can be stated that in the extra-foveal regions which have so far been studied, the square root law of temporal summation applies when $d < d_0$ and $t > t_0$, the square root law of spatial summation applying when $d > d_0$ and $t < t_0$.

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An Improved Technique for Intravenous Injection of Newborn Rats and Mice

The intravenous injection of neonatal rats and mice is an exacting procedure; any refinement of technique which permits a safer, more reliable and more accurately measured infusion should be of value. In this laboratory a method has been evolved which has some of these advantages.

Using a simplified and inexpensive continuous slow injection apparatus (Fig. 1), designed and built by the Department of Medical Physics at a cost of less than £10, solutions or cell suspensions may be delivered at a slow and uniform rate in precisely measured quantities.

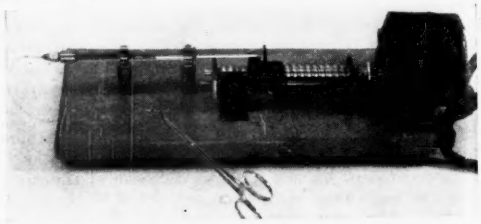

Fig. 1. The apparatus consists of a synchronous motor driving a lead screw which in turn activates a 'ram'. The ram travels along the length of the lead screw depressing the plunger of the syringe. The lead screw is coupled to the motor by a sprung gear wheel, in order that the ram may be started at any position on its travel, depending on the amount of fluid in the syringe. A micro-switch disconnects the supply to the motor when the ram has travelled its full distance to prevent breakage of the syringe. The sprung gear wheel arrangement allows the ram to be re-wound by hand, up the lead screw, ready for the next injection

Fig. 2

A 30-gauge unmounted needle with a short bevel is connected by 8 in. of polyethylene tubing to a 1- or 2-ml. syringe fixed between anvil and ram of the slow injection apparatus. The needle may be held in a hæmostat with one hand, the animal immobilized with the other hand (Fig. 2), and the delivery of the dose controlled by a foot switch to the motor. In this way the operator may direct his full attention to the insertion of the needle and to its fixation throughout the period of injection. It is also possible to carry out the injection single-handed if instead of using an assistant to read volume injected from the syringe, a sound signal (for example, a simple metronome or time alarm) is used to mark the time equivalent previously determined for the required volume. A number of animals may be injected without refilling the syringe and volumes up to 0·1 ml. may be safely given in less than one minute. It is however advisable when injecting cell suspensions to rotate the syringe between injections to minimize sedimentation.

The injection is made through the lateral canthus of the unopened eyelids and into the subcutaneous vein at the junction of the superior and inferior palpebral veins. These veins may be more easily discerned if the skin is wiped with liquid paraffin and the thorax is gently compressed with the restraining finger to increase central venous pressure and to distend the veins at the moment of venupuncture. With the adoption of this method the injection failure rate has fallen from 30 per cent to about 5 per cent.

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PLANT PHYSIOLOGY

The Organic Acids in Rose Hips

As part of a general investigation into the physiology of rose hips, the organic acid content of the flesh was followed during development in the species *arvensis*, *canina*, *rubiginosa*, *Sherardi* and *rugosa*. The first four indigenous species were chosen to cover the wide range of ascorbic acid concentration found in the flesh of different species growing in one area[1,2], while *rugosa* was included because other aspects of its physiology were under examination[3,4].

It had already been demonstrated that the main acids present were citric, malic and quinic, together with traces of succinic acid[5]. The spot-area method of chromatographic estimation was then used to follow quantitatively the changes in amount; at the 95 per cent level of probability the error was less than ±10 per cent, and other microchemical and microbiological methods were used to confirm the results[2].

Since the bulk of cell division in the flesh of a fruit is usually completed very early in its life[6], analysis was begun as soon as possible during development so as to obtain information about this stage in its history. Samples were taken as follows, that is, at intervals of approximately three weeks: Stage I: very tiny flower-buds usually 3–4 mm. in diameter; stage II: flower-buds just opening; stage III: about 7–10 days after petal-fall; stage IV: full-size, green hips; stage V: firm, red hips at the peak of physiological maturity; stage VI: over-ripe hips which are softening but not damaged.

As an example, Fig. 1 gives the results for *canina* on a dry-weight basis. This shows that at stages I and II (when cell division is active), malic and quinic are the main acids; after this the position in relation to citric acid is reversed and the latter becomes the principal acid at maturity. Since the interconversion of malic acid and citric acid by the hip flesh had been demonstrated[2], the fall in the concentration of malic acid which accompanies the increase in citric acid might seem to indicate that some of the latter had arisen in this way. If the results are expressed on a 'per hip' basis, however, the fall in malic acid is eliminated and it seems probable rather that the synthesis or import of citric acid, and other materials comprising the dry matter, had outstripped the accumulation of malic acid.

Estimations of the soluble, protein and total nitrogen were also made, and the values for the organic acids re-calculated on a protein basis. The most obvious change is the removal of the peak in the total organic acidity at stage II. This is because of the relatively large proportion of protein at stages I and II, so that when the results for these stages are expressed on a protein basis they are reduced by comparison with the figure for stage III and the peak

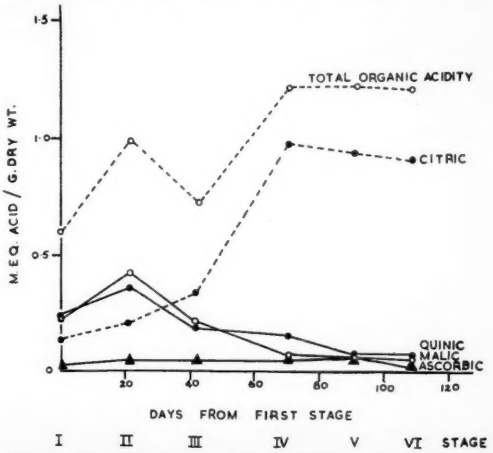

Fig. 1. Changes in the concentrations of total organic acidity and of the individual acids during the growth of hips of *R. canina*, expressed as milli-equivalents /gm. dry weight

disappears. The same change occurs in the patterns for malic acid and quinic acid.

The results on a protein basis also show that, in terms of cellular growth, it seems likely that there is little change in the concentration of citric acid in the protoplasm of the dividing cells (stages I and II). Once vacuolation begins in earnest (stage II), the amount of acid increases steadily to maturity, which suggests that it is to be found predominantly in the cell sap rather than in the protoplasm. The pattern for ascorbic acid is almost identical although at a much lower level of concentration and, indeed, this similarity in trends is the one positive correlation between the carboxylic acids and ascorbic acid.

On the other hand, the concentrations of malic acid and quinic acid on a protein basis are (relatively speaking) so constant as to invite the conclusion that they are components of some cytoplasmic systems. Mapson[7] has suggested that dihydroshikimic acid, shikimic acid and quinic acid may form a dehydrase system similar to the succinic-fumaric-malic acid one, and Weinstein *et al.*[8] have shown that rose petals of a commercial variety can form shikimic acid from quinic acid labelled with carbon-14. So far as malic acid is concerned, results will be presented shortly for respiratory measurements and feeding experiments with tissue strips of hip flesh, which are consistent with the operation of at least part of the Krebs cycle in this false fruit.

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Cytochrome System of *Streptomycetes*

It has recently been reported that the cytochrome system in the genus *Streptomyces* differs strikingly from that found in most aerobic cells[1]. No *a*-type cytochrome could be seen in the 10 species examined, and in some cases cytochrome *c* was also apparently missing. The suggestion[2] that the respiratory chain may terminate with electron transfer from cytochrome *b* to oxygen was further supported by the partial purification of an autoxidizable cytochrome *b*. Since a similar '*b*-oxidase hypothesis' has been invoked to account for the cyanide-resistant respiration in higher plants[3,4], the Streptomycetes were re-examined with the view of shedding some light on this problem. However, a survey of representative *Streptomyces* species showed that their respiration can be inhibited by cyanide and further studies have shown that *a*-, *b*-, and *c*-type cytochromes are present in all cases.

Mycelium was grown in a liqiud medium ('Bacto' nutrient broth, glycerol, *p*H 7) on a rotary shaker at 30° C. The endogenous respiration of *S. coelicolor*, *S. fradiae*, *S. lavendulae*, *S. griseus* and *S. rimosus* pellets was inhibited between 40 and 70 per cent by $10^{-3}M$ hydrogen cyanide. In order to examine the respiratory components, cell-free extracts were prepared by treating a buffered (*p*H 7) mycelial slurry for 10 min. in a sonic oscillator (10 kc./3.). The particulate fraction was isolated by centrifuging at 80,730*g* for 1 hr., following preliminary centrifugations to remove cell debris, and the golden brown pellet was suspended in a small volume of buffer.

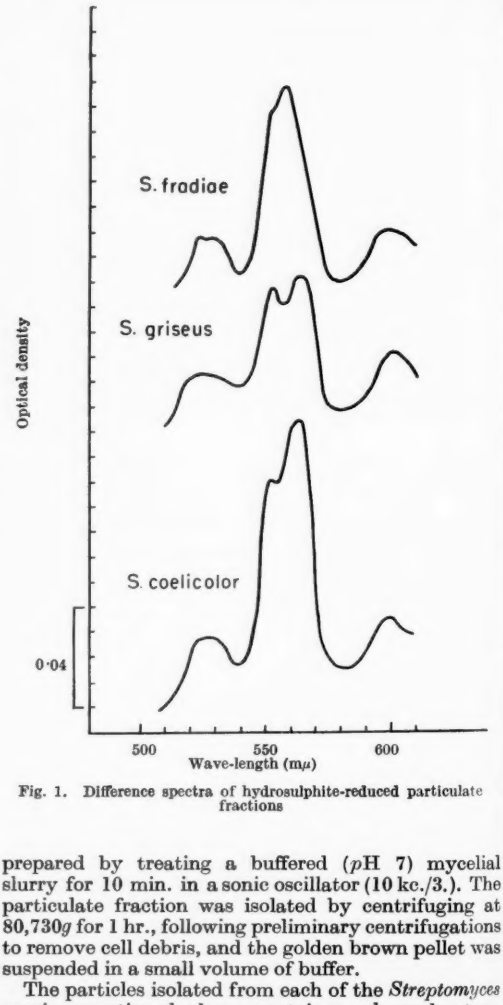

Fig. 1. Difference spectra of hydrosulphite-reduced particulate fractions

The particles isolated from each of the *Streptomyces* species mentioned above contain *a*-, *b*- and *c*-type cytochromes and typical difference spectra (reduced-oxidized) are shown in Fig. 1. After reduction by hydrosulphite the spectra show characteristic absorption maxima at 600 (*a*), 558–564 (*b*), and 550–552 (*c*) mμ, as well as a broad band at 520–530 mμ. It is clear that the relative amounts of the individual components differ markedly with the species. The *S. coelicolor* and *S. fradiae* particles contain unusually high concentrations of the *b*-type cytochrome, while in *S. griseus* the cytochromes are present in roughly equimolar concentrations. In the Soret region, the major absorption band shows a peak between 428 and 434 mμ, indicative of a *b*-type cytochrome; with *S. griseus*, this band has a shoulder at 445 mμ, corresponding to the γ-band of an *a*-type cytochrome. A trough in the 460–475 mμ region indicates the presence of flavoprotein.

The individual components were delineated more clearly using reduced diphosphopyridine nucleotide and succinate to reduce the respiratory chain. Fig. 2 shows the enzymatically reduced *S. fradiae* components, and, in contrast to the situation with hydrosulphite, the difference spectra are not dominated by cytochrome '*b*'. The α-bands of cytochrome *c* (552 mμ)

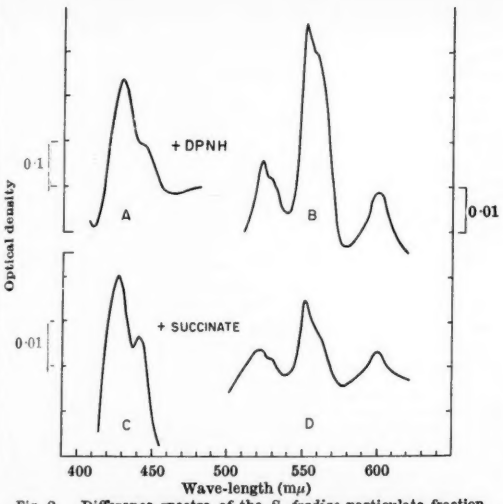

Fig. 2. Difference spectra of the *S. fradiae* particulate fraction reduced with reduced diphosphopyridine nucleotide or succinate. *A*, 10 mgm. protein; *B*, *C*, *D*, 0·6 mgm. protein/0·5 ml.

and *a* (600 mμ), as well as *b* (559 mμ), can now be readily distinguished; the Soret peak of an *a*-type cytochrome (440–445 mμ) can be seen in addition to the major 428 mμ peak. There is clearly a large amount of hydrosulphite-reducible cytochrom *b* which is not reduced in the respiratory chain. The reduced '*a*' component reacts with carbon monoxide, resulting in a decrease in absorbance at 440 mμ and the appearance of a new peak at 420–422 mμ, which may be compared to the bacterial carbon monoxide–binding pigments[5]. With *S. griseus* particles, the carbon monoxide spectrum shows a valley at 445 mμ and a peak at 428–430 mμ, thus corresponding more closely to the classical a_3-carbon monoxide complex.

Some evidence that the isolated particles represent the normal respiratory centres has been obtained. The particulate succinoxidase activity, like the oxygen uptake by intact mycelium, is inhibited 70 per cent by $10^{-3}M$ hydrogen cyanide and only slightly by 1γ/ml. antimycin *A*. The particles readily oxidize reduced diphosphopyridine nucleotide but show only slight activity with reduced mammalian cytochrome *c*.

In an attempt to reconcile our data with the report of Heim *et al.*[1], alkaline homogenates of *S. fradiae* were prepared and examined by their methods. Although cytochrome '*b*' could be seen in the homogenate, the concentrations of '*a*' and '*c*' were too low to permit detection until the particulate fraction had been isolated. Although the present experiments do not eliminate the '*b*-oxidase hypotheses', the evidence for a complete and functional cytochrome system must be carefully considered.

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Composition of Sieve-Tube Sap

Sap from the sieve-tubes of the willow *Salix viminalis* was obtained using the mouthparts of the willow aphid as described by Kennedy and Mittler[1] and us[2]. As Mittler[3] found, sucrose formed the main constituent of the total solids and so for routine concentration measurements, a refractometer calibrated directly in percentage sucrose was used. The sugar content was analysed by paper chromatography, the total nitrogen content measured using a micro-kjeldahl method[4] and the individual amino-acids present detected by paper chromatography.

These analyses confirmed those carried out previously by Mittler[3], Wanner[5], Ziegler[6] and Zimmermann[7]. Apart from sucrose the only other carbohydrates present were raffinose and stachyose. The former was found in quantities up to 15 per cent of the total carbohydrate present, the latter as a trace only. Traces of mannitol were present, but no methylated or phosphorylated sugars could be detected. As Mittler has shown, the amino-acid fraction varies with the time of year. During October–April we found some ten amino-acids to be present, but during the rest of the year no more than three: aspartic and glutamic acids and asparagine.

The concentration of the solutes in the sap also varied considerably with time of year. Lengths of stem taken from the field between mid-October and March and sprouted in the laboratory, yielded a sap with a sucrose concentration of 25 per cent, whilst the sap from summer cuttings was as low as 4–6 per cent. No further investigation of other sap constituents was undertaken until a large discrepancy was revealed between the measured osmotic potential of the sap and that calculated from the sucrose concentration based on the refractometer reading. The osmotic potential of the sap was measured using a vapour pressure method[8] by means of which quantities as small as 1 μl. could be used. Since the discrepancy often amounted to as much as 15 atm., the presence of some hitherto unknown osmotically active constituent seemed likely.

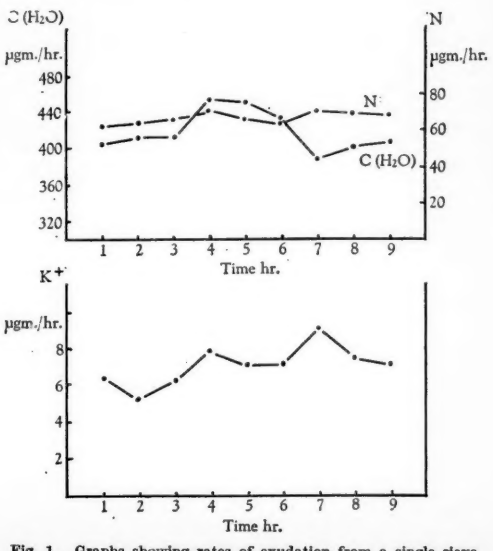

Fig. 1. Graphs showing rates of exudation from a single sieve tube of willow. Upper part, nitrogenous compounds (N) carbohydrates (CH_2O); lower part, potassium.

Attention was therefore turned to cations. Analyses using a flame photometer revealed traces of sodium, no calcium, but up to 2 per cent w/v of potassium. Clearly such a concentration of potassium could readily account for the observed osmotic discrepancy. In seeking the anion partner of the potassium, no nitrate could be detected using the phenol disulphonic acid method nor could chloride or phosphate using silver nitrate. The presence of organic acids was investigated by paper chromatography[9]. This revealed citric, tartaric and possibly oxalic acids. It is concluded that the potassium is present as the salts of these acids.

The rate of exudation (μgm./hr.) of the three main constituents of the sieve tube sap, sucrose, nitrogen and potassium has been measured over periods of hours. As shown in Fig. 1 there is no evidence for a decline or change in ratio between the concentrations of these three constituents. This, and its high rate of exudation would seem to preclude the possibility that the potassium is secreted into the sieve tube as a consequence of some local disturbance produced by the stylets. Rather is it moving along the sieve tube with the sugar and amino-acids in a normal manner.

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Effect of Oxygen on Endogenous Metabolism of a Respiratory-deficient Brewer's Yeast

In a previous communication, a strain of brewer's yeast was described which can utilize its carbohydrate reserves by a fermentative mechanism[1]. During this work it was observed that the presence of oxygen resulted in yeast carbohydrate being degraded at a faster rate. In this yeast, investigation of the effect of oxygen on the endogenous fermentation was hampered by oxidation of ethanol and liberation of respiratory carbon dioxide. A respiratory-deficient mutant provides a system wherein the residual respiration does not interfere to a significant extent. Experiments with one of these mutants have shown that oxygen stimulates the endogenous fermentation.

The respiratory-deficient mutant used for this work had been obtained by growing the experimental yeast in a medium[2] containing 0·006 per cent cupric sulphate ($CuSO_4\ 5H_2O$). The culture was plated on nutrient agar and the respiratory-deficient colonies were selected with the aid of the triphenyltetrazolium chloride overlay method[3]. The mutant strain selected had an initial endogenous Qo_2 (μl. of oxygen taken up per mgm. initial dry weight of yeast per hr.) of 1·0–3·5 compared with 12–14 for the parent yeast. The endogenous fermentation under anærobic conditions of cells of the same carbohydrate content was of the same magnitude as in the parent yeast.

The respiratory-deficient yeast was grown in the same way as described previously for the normal yeast, in standing flasks at 25°C., and harvested in the stationary phase of growth when the glucose concentration had fallen to about 1 per cent. The conditions of growth were substantially anærobic. The cells were rapidly washed three times and suspended in $M/15$ potassium dihydrogen phosphate (pH 4·5). During the harvesting and washing of the yeast, no special precautions were taken to exclude air.

The carbohydrate disappearing and carbon dioxide and ethanol produced under ærobic and anærobic conditions were compared in Warburg manometer flasks at 28°C. The contents of manometer flasks for carbohydrate determination were treated with an equal volume of M trichloro-acetic acid and the suspension diluted to a suitable volume for estimation with the anthrone reagent. The suspensions from the flasks for ethanol determination were rapidly centrifuged and the ethanol distilled from the supernatants. A method based on the reduction of diphosphopyridine nucleotide in the presence of liver-alcohol dehydrogenase was used for ethanol estimations[4]. Table 1 shows the results for a typical experiment. The greater carbohydrate breakdown under ærobic conditions was accompanied by the production of more ethanol and fermentation carbon dioxide. The fermentation carbon dioxide was calculated by assuming that carbohydrate was the substrate of the residual respiration. (Respiratory quotient = 1·0). Even making this assumption, the amount used up by respiration would account for less than 10 per cent of the extra carbohydrate degraded. The ethanol found was about 23 per cent less in both cases than

Table 1. Comparison of Endogenous Fermentation under Air and Nitrogen

Each manometric flask contained 3 ml. of yeast suspension. The initial dry weight of 3 ml. of suspension was 6·39 mgm. and the total carbohydrate content was 2·78 mgm. During gassing and equilibration of the flasks, the carbohydrate content per flask fell to the levels shown. The duration of the experiment was 150 min. Carbohydrate is expressed as the equivalent amount of glucose.

Gas phase	Oxygen uptake (μl.)	Fermentation carbon dioxide (μl.)	Carbohydrate equivalent of: Oxygen (mgm.)	Carbohydrate equivalent of: Carbon dioxide (mgm.)	Carbohydrate found (mgm.): After equilibration	Carbohydrate found (mgm.): 150 min.	Carbohydrate found (mgm.): Change	Ethanol found (mgm.): After equilibration	Ethanol found (mgm.): 150 min.	Ethanol found (mgm.): Change
Nitrogen	—	150	—	0·6	2·13	1·62	−0·51	0·23	0·43	0·20
Air	22·5	230	0·03	0·92	2·08	1·24	−0·84	0·25	0·58	0·33

Table 2. Changes in Rates of Oxygen Uptake and Carbon Dioxide Release with Time

The gaseous exchanges are expressed as μl. taken up or released per mgm. initial dry weight of yeast per hr. The initial dry weight of yeast per flask was 7·14 mgm. The carbon dioxide is not corrected for respiration.

Time (min.)	0–60	60–120	120–180	180–240	240–300
Rate of oxygen uptake:					
Air	1·8	1·5	1·1	1·0	0·8
100 per cent oxygen	3·6	2·6	1·8	1·3	0·9
Rate of carbon dioxide release:					
Nitrogen	14·5	7·0	4·9	3·3	2·6
Air	21·9	12·9	8·3	5·5	3·3
100 per cent oxygen	26·1	14·8	9·1	5·6	2·9

expected from the breakdown of carbohydrate. The low yields of ethanol might be due to its retention in the cell or its resynthesis into carbohydrate.

When 100 per cent oxygen was used in the manometer flasks instead of air, the rate of carbohydrate degradation was further increased and more carbon dioxide was released. The rate of oxygen uptake was also greater than the rate in air. Table 2 shows the time course of endogenous metabolism under nitrogen, air and 100 per cent oxygen. In this particular experiment, the carbohydrate breakdown per flask in the first hour was 0·42 mgm. in the flask under nitrogen, 0·59 mgm. under air and 0·67 mgm. under 100 per cent oxygen.

These results indicate that in the strain of yeast used in these experiments, endogenous fermentation is stimulated by the presence of oxygen, the stimulation increasing with increasing oxygen tension. The level of oxygen uptake rises with increasing oxygen tension but remains small. It is interesting to compare this effect on reserve carbohydrate with the well-known Pasteur effect, which refers to the inhibition of fermentation of external carbohydrate by oxygen and results in a sparing effect on the breakdown of carbohydrate.

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Carbohydrates of the Rampion, *Campanula rapunculus* L.

Despite such studies as those of Chollet[1] it does not seem to be generally recognized how closely the water-soluble carbohydrates of the Campanulaceae resemble those of the Compositae. For example, this resemblance is not mentioned in recent books by Bonner[2], Pigman[3], and Karrer[4].

Chollet showed that several species of *Campanula* contain fructose polysaccharides resembling those of the Jerusalem artichoke. We have recently examined extracts of the fleshy roots of another species, *C. rapunculus*, the rampion, and find that they contain a series of non-reducing oligosaccharides indistinguishable on paper chromatograms from those of composites[5]. Fractionation by gradient elution with aqueous ethanol from charcoal-'Celite' columns showed that only one trisaccharide was present (as in *Helianthus tuberosus*[6]). This was crystallized and, as with *H. tuberosus*, shown to be 1^F-β-fructosylsucrose; it had a fructose : glucose ratio[7] of 1·87 : 1, $[\alpha]_D^{15+}$ 27·6°, and the same infra-red spectrum as an authentic specimen.

When boiling-water extracts of the roots were left at room temperature for several days a precipitate appeared. This was a polysaccharide resembling inulin in its solubility, soluble in hot water (above 80°) but coming out of solution after freezing and thawing. However, it had $[\alpha]_D^{21}$ − 27° and a glucose content of 5·2 per cent; typical specimens of inulin have $[\alpha]_D$ at least −40° and 2–3 per cent glucose[8].

After removal of this polysaccharide, a further fructosan fraction could be precipitated by the addition of two volumes of ethanol. This had $[\alpha]_D^{21}$ − 29° (glucose content: 3·4 per cent), thus resembling that described as 'fructosane *B*' by Chollet[1], which he showed to be very similar to the 'inulénine' fraction isolated by Tanret[8] from *H. tuberosus*.

These findings would seem to argue a close evolutionary connexion between the Campanulaceae and Compositae. The review by Fuchs[9] shows that the Liliflorae, Glumiflorae and Compositae have been fairly extensively studied; but there is need for a survey, in the light of modern knowledge, and using newly developed analytical techniques, to determine the full extent of the occurrence of fructosans in the plant kingdom. Such a survey might cast useful light on some problems of classification[10], and also help to correct the present tendency to regard starch as the usual form of carbohydrate reserve in plants.

I wish to thank Dr. V. C. Farmer, of the Department of Spectrochemistry of this Institute, for examining the infra-red spectrum of the trisaccharide.

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RADIOBIOLOGY

Separation by Gamma-Irradiation of Two Elements at the V^{bv} Locus in *Trifolium repens*

The V^{bv} leaf-marking allele in *Trifolium repens* results in the production of a phenotype characterized by the presence of a broken white *V* surmounted by a yellow tip (Fig. 1*a*). A separation of the two parts by

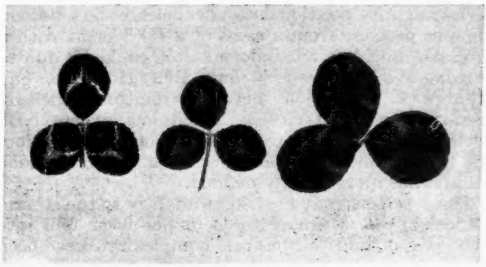

Fig. 1. Leaves of *T. repens*. *a*, Normal; *b*, yellow tip phenotype; *c*, white *V* phenotype

crossing-over has never been reported. There are eleven known alleles at this locus[1], of which *v* determining the absence, is recessive to all alleles determining the presence of a mark. Most compounds not involving *v* exhibit mosaic dominance[2].

Two clones heterozygous for V^{bv} and *v* were utilized in a series of experiments to determine the dose response to gamma-radiation, in terms of induced mutations at this locus, to examine the effect of dose-rate and dose fractionation (Davies and Wall, in press), and also to study the possible complex nature of the locus. Since the gene being investigated is already known to exist in allelic forms of varying expression, there seemed a good opportunity of detecting new phenotypic expressions. The locus is also advantageous in that the leaf marks probably have little physiological effect and mutants would not be at a disadvantage. The term mutation is used of necessity in its broadest sense—point mutations here cannot be distinguished from partial or whole chromosome losses.

Many mutant phenotypes were found after irradiating somatic tissue of the heterozygous material, but none was found in control material. The mutants included some in which either both or only one of the component parts of the mark had been lost (Fig. 1), and others which exhibited a modified form of mark. There were two of the latter types—one in which the white *V* was reduced to spots and another in which the white *V* was reduced in intensity. Of these mutants—the two modified, and the yellow tip (Fig. 1*b*) phenotypes do not, so far as is known, occur naturally. The relative frequencies of the loss mutations were:

Loss of whole mark	103
,, white *V* (Fig. 1*c*)	80
,, yellow tip (Fig. 1*b*)	18

These values refer only to those instances where a whole leaflet or larger portion of tissue was mutant, and do not include smaller mutant areas where there could be some uncertainty regarding the exact nature of the loss. It is impossible to quote a mutation-rate in an instance such as this where the number of potential sites of mutation is unknown; but some indication of the frequency of the effect is given by the percentage of leaves exhibiting mutations. After exposing somatic tissue to 4,000 rads, there were 3·97 per cent mutant leaves.

Though the recombinational test in *Trifolium repens* is extremely poor, it is surprising that no recombinants have been found, in view of the comparative ease with which the two parts of the phenotype were separated by radiation. It is conceivable that cross-over suppressors are present.

The separation has indicated the structural complexity of the V^{bv} locus. This is not unexpected in cases of multiple allelism where the alleles do not produce simple quantitative differences. Brewbaker[2] has suggested that at least some of the leaf-marking alleles might be positional pseudoalleles, as he obtained some *vv* progeny from a cross of a VV^B plant with a *vv* male. Such an anomalous result could be due to crossing-over between *V* and V^B. However, any attempted distinction between multiple allelism, pseudoallelism and closely linked loci, where both alleles are usually manifested in the heterozygote, does not have much meaning, until allelism in such instances is more strictly defined.

The V^{bv} locus may bear some analogy to the *A*[3] and R^4 loci in maize where the locus has been split into two parts, and structural complexity has been demonstrated to be almost certainly associated with chromosomal duplication. There the homologous repeats are separable, at a very low rate, by crossing over.

It is quite likely that in the case of the V^{bv} locus, as in most instances of mutations in maize, the mutant phenotypes are associated with chromosomal changes. In this instance the changes are probably deletions and the high rate of occurrence would tend to substantiate this. If this is the case, and assuming the linear order on the chromosome of the sites of action for the two parts to be centromere (*C*), yellow tip (*T*), white *V* (*W*), then the distance *C* to *T* is of the same order as *T* to *W*, since the rate of loss of the whole mark is similar to that of the white *V*. However, if viable deletions are confined to terminal deletions, then the distance *C* to *T* could be much greater than *T* to *W*. A two-hit aberration would be required to eliminate *T* alone.

The V^{bv} mutants produced may have some practical interest, in connexion with problems of seed certification. The problem of authenticity in clovers is complicated, and strain marking using leaf-marks is being undertaken[5]. It is conceivable that some of the artificially induced leaf-marks could contribute to a solution of the problem. As the mutant tissue was completely viable, and wholly mutant tillers and plants were produced, a crossing programme was undertaken to test the mode of inheritance of the new phenotypes. However, all the mutants proved to be chimaeric and the flowers were all derived from normal tissue. In view of the possible practical value of the new leaf-marks, a further programme is now being undertaken, in which seed heterozygous for V^{bv} and *v* is being irradiated in an attempt to produce mutant tissue which will not be chimaeric.

We are indebted to Prof. K. Mather for guidance in the preparation of this report.

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Biological Action of Potassium-40 in Naturally Occurring Potash Salts

With regard to the radiation effect of naturally occurring radioactive substances, Rahn[1] was unable to establish any effect on the growth of micro-organisms due to the radioisotope potassium-40 present in normal potassium. Likewise, Vinogradov has investigated the reactions of the heart muscles in the frog[2] and the growth processes of *Aspergillus niger*[3,4] to potash salts enriched with potassium-40 without being able to demonstrate a radiation effect specific to potassium-40. In the experiments with *Aspergillus niger* salts with high (1·34 per cent), and low (0·002 per cent) potassium-40 content, in addition to normal potash salt (potassium-40 content = 0·0119 per cent) were used.

The conclusion drawn by Vinogradov from his experimental results that the radioactivity of potassium-40 does not play any part in the growth of *Aspergillus niger* appears to us to be insufficiently assured in view of the differences in weight of the small doses of potassium.

We have therefore re-examined the possibility of an effect of potassium-40 in the range of potassium-

deficiency and carried out appropriate experiments with the yeast *Torula utilis*. In preliminary experiments the course of the growth-curve was first determined, in addition to the requirement for normal potassium under the conditions chosen for the later experiments. The culture solution ($M/150$ sodium phosphate buffer of *p*H 6·8) had the following composition: Glucose (5 per cent), ammonium nitrate (0·75 per cent), magnesium sulphate (0·05 per cent).

Potassium chloride was added at four levels of concentration, namely, 1×10^{-4}, 5×10^{-4}, 1×10^{-3}, and 5×10^{-3} in terms of molarity.

The work was performed with 50 ml. of nutrient solution in 200-ml. Erlenmeyer flasks, and with 4 parallels at a time. After inoculation the culture flasks were shaken for 3 days on a shaking machine having a horizontal motion (150 cycles per minute); finally, the yeast suspensions were filtered and the dry matter obtained by freeze-drying. As can be seen from curve *a* in Fig. 1, the greatest mass of dry matter was produced by the 5×10^{-3} M potash salt treatment. No further increase of the dry matter was obtained by strengthening the given solution.

In order to counterbalance the osmotic influence exerted by the different concentrations of potassium chloride, and also to establish the effect of a possible replacement of potassium by sodium, a second supplementary experiment was carried out at the same time at the same levels of salt concentration. For this purpose the low additions of potash were brought up to 5×10^{-3} M by the addition of sodium chloride (Fig. 1, curve *b*). The course of formation of dry matter shows that the addition of sodium chloride has produced an increased growth of yeast. This effect diminishes with increasing additions of potash and approaches zero at the optimum concentration of potash of 5×10^{-3} M.

In addition to these two incremental series of potash with, and without the addition of sodium chloride a third experiment was carried out with enriched potassium-40. The salt, enriched to the extent of 46·6 times, was obtained from Harwell and was used at a concentration of $1{\cdot}0 \times 10^{-4}$ molar total potassium chloride, without and with the addition of sodium chloride. The yeast dry matter formed is shown by the point on both curves.

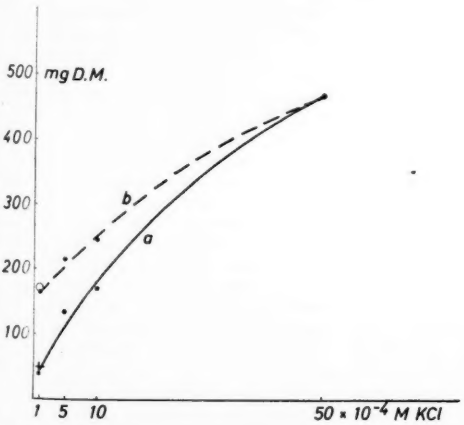

Fig. 1. Effect of normal and potassium-40 enriched (46·6-fold) potassium chloride on growth of *Torula utilis* (yield in mgm. dry matter). *a*, potassium chloride without sodium chloride; *b*, potassium chloride with sodium chloride; +, potassium chloride enriched with potassium-40 but without sodium chloride; O, potassium chloride enriched with potassium-40 and with sodium chloride

Since the work was conducted in a range of concentration in which the growth-stimulating effect of potassium is sharply marked, a radiation effect on the formation of substance was here first to be expected. However, the results show no values of dry matter falling below the normal range of variation in comparison with those obtained at the same levels of concentration of normal potassium chloride.

Thus, in agreement with Vinogradov, it has been shown that no measurable effect on growth results from the radioactivity of potassium-40 in the case of *Torula utilis*.

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Relative Rates of Exchange of Potassium-42 by the Fœtal Metanephros and Fœtal Liver

Walker *et al.*[1] and Ginsberg *et al.*[2] have shown that the kidney of the adult rat exchanges potassium-42 at higher rates than any other organ. After 2 min. the radioactivity of the kidney is eight times that of the liver[2]. We have studied the relative rates of exchange of potassium-42 in the liver and kidneys of 24 rat fœtuses at the 20th or 21st day of development. 100 μc. of potassium-42 were injected into the tail vein of twelve pregnant rats at the 20th or 21st day of pregnancy and 2 min. later two fœtuses were removed and the kidneys and liver excised. These organs were frozen with cardice to prevent dehydration, weighed to 0·1 mgm., digested in concentrated nitric acid and their radioactivity determined. The results were expressed as the ratio of the number of counts of potassium-42 per min. per mgm. of fœtal kidney to the number of counts of potassium-42 per min. per mgm. of fœtal liver. The total amount of potassium per mgm. of fœtal kidney and liver was also estimated in six fœtuses using a Warren flame spectrophotometer[3].

The results of the radioactivity determination showed that the rate of exchange of potassium by the fœtal kidney was twice that of the fœtal liver (Table 1). There was no significant difference in the total potassium content of the liver and the kidney.

Table 1

The relative uptake of K^{42} by the fœtal liver and kidney of the rat 2 minutes after I.V. injection into the mother at 20th and 21st day of pregnancy.

Age of fœtus	Number	*R ± S.E.
20 days	14	1·95 ± 0·1
21 days	10	1·86 ± 0·12

*R = The ratio of the counts of K^{42}/min./mgm. of fœtal kidney to the counts of K^{42}/min./mgm. of fœtal liver.

The precise functional significance of the high exchange rate of potassium in the adult kidney is not yet understood, and has not been demonstrated for any other organ. To investigate the problem further we decided to determine the developmental stage in which this high rate of exchange first becomes evident and to relate this to the histogenesis of the kidney. We chose as a starting point the late fœtal kidney in view of experimental evidence that it is functioning both with regard to glomerular filtration and tubular reabsorption[4,5], and urethral ligation in the fœtal rat results in a dilatation of the urinary tract[6,7].

These results show that the rate of exchange of potassium-42 by the late fœtal kidney is almost twice that of the fœtal liver, but this ratio is considerably lower than in the adult. The stage at which the fœtal kidney first begins to exchange potassium more rapidly than the fœtal liver therefore occurs earlier than the 20th day of pregnancy, but the greater part of this capacity for a high rate of exchange must appear during post-natal life. We found that there was no significant difference between the total potassium in the fœtal kidney and the fœtal liver and therefore the amount of radioactive potassium per mgm. of tissue is expressed directly, and not as the specific activity.

Moog[8] regarded the alkaline phosphatase as a reasonable indicator of kidney function. Alkaline phosphatase is largely restricted to the proximal tubules, and these have been shown to be the site of highest radioactivity in the adult rat kidney 2 min. after intravenous injection of potassium-42[9]. A histochemical study of the 20-day rat fœtus has failed to show the presence of alkaline phosphatase in the kidney.

We wish to thank Mr. R. Ekins for the radioactivity measurements and Mr. R. Warren for the total potassium determinations.

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Effect of Ultra-Violet Irradiation on Transduction in *Pseudomonas aeruginosa*

In 1958, I described a transduction-like process within a single strain of *Pseudomonas aeruginosa*[1]. The genetic transfer was described as transduction-like because it occurred when a mutant of *Pseudomonas aeruginosa* strain *B* was grown in the culture filtrate of another mutant or the wild-type organism itself; in addition the genetic material was unaffected by deoxyribonuclease and was associated with a particle greater than 100 mμ. in diameter. It was not possible to demonstrate that the particle was a lytic bacteriophage because no susceptible strain had been isolated.

Quantitative studies of the particles capable of transducing single markers showed that they were few in number and ranged from 3 to 8 per ml. of culture filtrate. Attempts to increase the number of particles were generally unsuccessful. A slight increase was obtained following small doses of ultra-violet irradiation and figures of up to 30 per ml. were obtained using the methods of Jacob[2]. However, the organisms can be regarded as non-inducible and the numbers produced were still not sufficiently high for further studies to be made as to the nature of the transducing particles.

In one experiment where much larger doses of ultra-violet irradiation were given to a culture of the wild-type organism, it was noticed that there was a much greater increase in the transducing activity of the filtrate than had been demonstrated for the smaller doses used in an attempt to induce lysis. The increase was initially assumed to be due to a process similar to induction, but subsequent experiments showed that the increase occurred whether the whole culture or the culture filtrate from a porosity 5 sintered glass filter was irradiated.

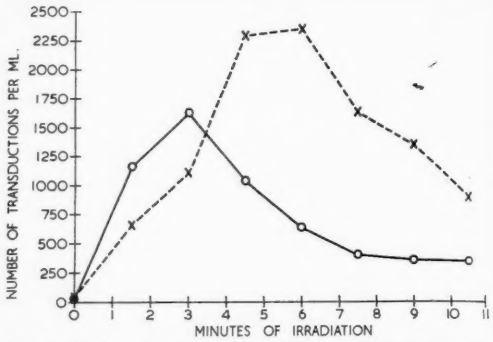

Fig. 1. Showing increases in transduction following ultra-violet irradiation of a whole culture (×) and a culture filtrate (O) of a strain of *Pseudomonas aeruginosa* which produces transducing particles

Fig. 1 shows the increases in transducing ability of preparations obtained following ultra-violet irradiation of both a whole culture and culture filtrate of strain 3*B*. This strain was derived from the wild-type strain *B* by continuing cultivation of strain *B* in a calcium-free synthetic medium. It produces 20–30 transducing particles per ml. of culture filtrate when grown overnight in dilute nutrient broth (4 gm./l. of Difco Nutrient Broth) at 39° C. The organism was grown in 1-oz. bottles containing 8 ml. of dilute broth and the cultures were pooled after incubation. The pooled material was divided into two parts and half of the suspension was centrifuged and filtered through a porosity 5 sintered glass-filter. This filtrate and the remaining culture was irradiated for 10½ min. and samples were removed at 0, 1½, 3, 4½, 6, 7½, 9 and 10½ min. Irradiation was carried out using 8 ml. samples in open 9 cm. Petri dishes which were agitated at a distance of 9 in. from a Westinghouse Sterilamp (*WL* 782–30). In the absence of any measurement of the output of this lamp some idea of its activity can be given by its killing action on bacteria. Cultures of *Pseudomonas aeruginosa* and also *Escherichia coli* strain *W* showed approximately a 1,000-fold kill in one minute under the same conditions. After irradiation, the whole culture samples were treated with chloroform for 2 hr., centrifuged and the clear supernatant decanted. Air was bubbled through this material at 37° C. for 20 min. to remove the chloroform.

To demonstrate the transducing properties of these preparations 1·0 ml. of the irradiated material was mixed with 0·5 ml. of an overnight broth culture of a leucine-requiring mutant derived from the wild-type strain *B*, and 0·5 ml. of nutrient broth. Equal samples of 0·2 ml. from each mixture were spread on minimal agar plates on which the mutant could not grow, the plates were incubated for 48 hr. and the colonies which developed were counted. Control plates spread with the mutant cells alone showed no colonies at all after incubation.

The results from the whole culture and the filtrate preparations show two major differences. Irradiation of the whole culture produced a preparation which gave a greater number of transductions per ml. than did the irradiated filtrate. The greater number does not necessarily reflect a greater effect due to ultra-violet irradiation since the proportionate increase

seems to have been approximately 70-fold in each case. It probably reflects the initial loss of particles from 33/ml. to 22/ml. due to filtration. There is also a difference between the irradiation times necessary to produce maximum increase. The maximum effect was observed in 6 min. when the whole culture was irradiated and 3 min. when the filtrate was irradiated. This difference is possibly due to the masking effect of the bacterial cells during irradiation of the whole culture. The activation obtained is very much greater than that shown by Garen and Zinder[3] using bacteriophage from *Salmonella typhi-murium*. They demonstrated approximately a six-fold increase in transduction following ultra-violet irradiation.

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Synthesis of Deoxyribonucleic Acid in Adult *Drosophila*

It is well known that thymidine labelled with hydrogen-3 provides a very accurate means for mapping synthesis of deoxyribonucleic acid in cell nuclei[1]. In an attempt to confirm by this method earlier conclusions drawn from work with phosphorus-32 regarding the length of time required for the maturation of sperm in *Drosophila melanogaster*[2], observations were made on synthesis of deoxyribonucleic acid in the fly in general.

Two types of experiment were performed. In the first, flies aged less than 3 hr. after eclosion were injected with about 0·05 μl. labelled thymidine (Schwartz Laboratories, New York) of about 0·9 mc. per ml. (and about 180 mc./m*M*). Flies were killed at daily intervals, up to 9 days after treatment. On the basis of findings in this experiment, a second series was performed where flies were similarly injected at from less than 2 hr. to 3 days after eclosion, and killed 24 hr. after treatment. The distribution of tritium was studied by means of stripping film autoradiographs[3], made with Kodak *AR*–10 over 5–7 μ paraffin sections. The exposure time was about three weeks.

In the first series, positive autoradiographs were found over nearly all nuclei in the imaginal fat body, showing that these cells continue their synthesis of deoxyribonucleic acid for at least some hours after eclosion. Positive reaction was also found over the nuclei of large sections of the midgut. Exact grain-counts were not made, since the range of the tritium β-particles is too short for quantitative studies in sections; but over many nuclei 25–30 grains above background were seen. Muscle cells, brain cells, pericardial cells, gave no arginine nor did all but a very few cells in the Malpighian tubules, the ectodermal fore-gut and hind-gut, and accessory glands of the testis. The primary and secondary spermatogonia gave weak autoradiographs in flies killed during the first day or two after eclosion, and this could be followed with difficulty in secondary spermatogonia and spermatocytes on the following days, but definite grain accumulation over spermatids and sperm was not established.

In the second series, positive autoradiographs of the same magnitude (25–40 grains per nucleus) were seen over gut and fat body cells in groups of flies injected during the first 3 hr. after eclosion. The grain count over fat body nuclei in flies injected 9 hr. after eclosion is about the same; but in fewer nuclei, while the number of labelled cells in the gut appears to be the same as in younger flies. At 12 hr. after eclosion few nuclei in fat body and mid gut show autoradiographs, and at 24 hr. the grain count is in both tissues down to about 10–15 in the few remaining positive nuclei. The somatic tissues of flies injected 3 days after emergence give negative reaction. In this series, autoradiographs over primary and secondary spermatogonia were seen to be much stronger in flies injected 24 hr. or 3 days after eclosion, than in flies treated 2 or 3 hr. after eclosion.

These results show that there is a considerable amount of synthesis of deoxyribonucleic acid going on in the somatic cells of the fly during its first 12 hr. of adult life. This leads to a competition for the labelled precursor so that the tagging of the germ cells is not nearly as heavy as would be expected. In experiments where a limited amount of precursor is administered for the purpose of incorporation into the deoxyribonucleic acid of germ cells, in physiological or genetical studies, it therefore appears more efficient to use 24 hr. old flies, than newly ecloded ones.

On the other hand, this material (or possibly one of the larger species of Diptera, for example, *Musca domestica*) should provide a very useful tool for biochemists interested in the effect of different agents, for example, radiation, on the process of metabolism of deoxyribonucleic acid, since there are no mitoses in these cells, the synthesis of deoxyribonucleic acid most probably being connected with the establishment of polyteny of the fat body and mid-gut chromosomes. In some other often-used materials, such as rat small intestine or regenerating liver, the influence of the agent to be tested on the process of cell division may lead to difficulties in the interpretation of data[4].

The results also serve to demonstrate that in some respects the adult *Drosophila* is not necessarily a fully developed, static system, and this may be of importance in some experiments.

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BIOLOGY

Discrimination Between Ebb and Flood Tide in Migrating Elvers (*Anguilla vulgaris* Turt.) by Means of Olfactory Perception

In a previous communication on the behaviour of elvers in the Dutch Wadden Sea[1] it was pointed out that the animals most probably use the tidal streams for their migration from the open sea to inland water. They are carried inward by the flood at higher water levels, and go down to the bottom during the ebb tide so that they are not carried back seaward. This means that elvers should be able to discriminate between ebb and flood tide. The suggestion was made that this discrimination was based on changes in salinity during the tidal cycle.

In laboratory experiments, however, no conclusive preference for fresh or brackish water over sea-water was found. In part of the experiments the elvers

showed indifference with respect to salinity; in other cases they showed a preference either for fresh water or for sea-water. Tap water, sea-water and mixtures of both were used.

Contrary to their indifferent behaviour as to salinity, the elvers showed a resolute preference for natural fresh water originating from inland sources such as the IJssel Lake and the Noord Hollandsch Kanaal. Even when natural fresh water was mixed with sea-water it was preferred to pure tap water by the elvers. After treatment with a charcoal filter, however, the natural fresh water lost its attractiveness. When in experiments a preference was found for lower or higher salinities, this could in the majority of cases be ascribed to a factor that could be removed by a charcoal filter.

Consequently, there can be no doubt that the preference for inland water is based on olfactory stimuli, while differences in salinity are of little importance.

In this connexion it should be mentioned that Neubaur[2] believes that elvers, during their migration in the Nord-Ostsee-Kanal, are guided by the smell of food; his assumption is based on the fact that elvers largely avoid tributary brooklets with clear fresh water and prefer the main canal with brackish water.

Further the work of Teichmann[3] may be mentioned, who demonstrated the remarkably high sensitivity of the olfactory sense in the eel.

The finding that smell is of so much importance rendered it necessary to modify the hypothesis on the discrimination between ebb and flood tide. It is now supposed that during the ebb tide, when inland water smell increases, elvers stay near the bottom, and during the flood tide, when the inland water smell decreases, they rise to higher water-levels.

This new hypothesis was tested experimentally in a transparent circular channel (15 cm. wide, 30 cm. high, outside diameter 80 cm.), in which a current was generated by means of rotating paddle-boards.

Experiments were performed with different current velocities (10, 22, 35, and 49 cm./sec. at 15 cm. above the bottom). In order to imitate the ebb stream the composition of the water was gradually changed from pure sea-water (1·7 per cent chlorine) to a mixture (of about 1·0 per cent chlorine) of sea-water with natural untreated inland water. The flood stream was imitated by a gradual return to pure sea-water.

During the experiments with weak and moderate currents (10 and 22 cm./sec.) the elvers reacted positively rheotactically to the 'ebb stream' and negatively rheotactically to the 'flood stream'. No differences, however, were found in numbers of elvers rising to higher water levels.

During the experiments with strong currents (35 and 49 cm./sec.) the elvers reacted to the 'ebb stream' by clinging to the bottom behind the small sand ridges formed by the strong current. Only a few of them rose to higher water levels. On the other hand, during the strong 'flood stream' the majority of the elvers rose from the bottom and a distinct tendency to swim to higher water levels was observed.

The high current velocities are comparable with those occurring in the Dutch Wadden Sea, where at the surface tidal streams reach a velocity of 150 cm./sec. ('Texelstroom') which corresponds with a velocity of about 1 m./sec. at 15 cm. above the bottom (depth 20 m.)[4].

In similar experiments in which the ebb and flood tide were imitated by salinity changes only, brought about by mixing tap water with sea-water, no differences in behaviour were found.

This evidence suggests that elvers are guided from the open sea to the inland water by olfactory cues, like adult salmon are guided to their parent streams[5,6]. However, whereas in the salmon the knowledge of these cues seems to be acquired by conditioning, in the elvers it is most likely to be inborn.

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An Unusual Diatom from the Antarctic

During the cruise of M.V. *Magga Dan* under charter to the Antarctic Division of the Australia Department of External Affairs during January-April, 1959, phytoplankton samples were collected by a modified Hardy Indicator, and later examined at the Commonwealth Scientific and Industrial Research Organization's Division of Fisheries and Oceanography, Cronulla. In the course of this examination, a peculiar diatom was observed on two occasions in the same sample. In each case, one valve of a cell had the characters of *Coscinodiscus*, the arrangement of the areolae resembling that of *C. lineatus*; but the valve was deeply concave. The other valve was characteristic of *Asteromphalus roperianus*. In one example two cells were in contact, one having the mixed character described, the other being a typical *Coscinodiscus*, with both valves identical. The other example was solitary, and smaller (65μ compared with 100μ), showing that it belonged to another generation.

The sample of phytoplankton contained a large number of cells of the *Coscinodiscus* and the *Asteromphalus* which were represented in the two valves of the aberrant cells. Fig. 1 shows the girdle view of the two cells, the concave nature of the *Coscinodiscus* valves, and the different shape of the *Asteromhalus* valve; Fig. 2 shows the *Coscinodiscus* valve with the hyaline rays of the other valve just visible below, and Fig. 3 shows the *Asteromphalus* valve with the *Coscinodiscus* valve below. It is interesting that the one valve is areolate, the other punctate.

Since the two aberrant cells represent two 'families' of the Diatomaceae, they create a considerable taxonomic difficulty and cast doubt on the phylogenetic significance of 'families' in this group of organisms.

Fig. 1. Girdle view of two cells, the lower with two *Coscinodiscus* valves, the upper with one *Coscinodiscus* and one *Asteromphalus* valve.

Fig. 2. Valve view of *Coscinodiscus* valve with outline of *Asteromphalus* valve showing through

Fig. 3. Valve view of *Asteromphalus* valve with *Coscinodiscus* valve below

Wood *et al.*[1] have previously reported a diatom cell with the characters of *Coscinodiscus lineatus* and *C. radiatus* in opposite valves, and inter-specific variants have been seen by other workers (N. I. Hendey *in litt.*), but such variations could be explained as inter-specific crosses. Several Antarctic *Actinocyclus* and *Schimperiella* species have two dissimilar valves, but this character is not characteristic of either *Coscinodiscus* or *Asteromphalus*. It is perhaps fortunate that the aberrant cells were observed as further division could have resulted in apparently normal *Coscinodiscus* and *Asteromphalus* cells, in which their derivation would not have been apparent. The fact that one cell of the pair was a normal *Coscinodiscus* makes an explanation of the phenomenon difficult.

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A Rare Hydromedusa

In June of this year a small medusa appeared in a salt-water aquarium tank at Aberdour, Fife. Stones placed in this tank had been gathered on the shore and dredged from below low-water mark at Aberdour. These stones had unidentified hydroids attached. Other medusæ appeared during June and July.

These medusæ have been identified as *Gonionemus*, probably *murbachi*, which Dr. F. S. Russell suggests may possibly be the same as *vertens*.

It is interesting to note that *Gonionemus* has only been recorded in Great Britain from aquarium tanks, once from the Laboratory at Cullercoats, once from the Laboratory at Port Erin and now from a private aquarium at Aberdour.

Specimens of this medusa have been sent to the British Museum (Natural History) and registered as No. 1959.9.2.1–9, and others are held by me.

Efforts are now being made to identify the hydroid.

Another medusa of interest appeared off Aberdour in June this year, when several specimens of *Eutonina indicans* were taken in a hand net from the sea at low tide.

I am indebted to Dr. F. S. Russell for identifying the *Gonionemus*.

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PATHOLOGY

Inhibition of Rous I Sarcoma Metastases by a Copper-Catalyst Blocking Agent

A connexion has previously been demonstrated between the activation of copper-enzyme catalysis and the production of tanned lipoprotein surface membranes[1]. These same membranes, under various experimental conditions, were found to be non-adhesive[2], and since tumour cell surfaces display this property[3], it suggested the possibility that cellular adhesion may depend on the integrity of a proportion of the protein moiety of the lipoprotein complex of the surface membrane.

When a tanned (sclerorized) membrane is treated with Mallory stain, it takes up the orange *G* dye to the exclusion of the other competitive aniline blue dye. Naturally occurring untanned membranes, or tanned membranes pretreated with chlorodioxyacetic, an agent which breaks down aromatic bonds in tanned structures[4], take up the aniline blue of the same stain. It is well known that the scleroprotein group, which includes keratin, myosin, and fibrinogen besides tanned protein, has an affinity for the orange *G* of Mallory stain, whereas the collagen and secretory group of proteins react with the aniline blue.

The Mallory staining of cells revealed that dried and methanol fixed smears of Rous 1 solid sarcoma and ascites cells, as well as red blood cells, show an affinity for orange *G*. These same cells after momentary pretreatment with the above agent, very much diluted, took up the aniline blue. The counterparts of the Rous cells, connective tissue cells and white blood cells (monocytes) respectively, show an affinity for the aniline blue. This differential staining has also been tried on hamster kidney tumour cells and their normal counterparts. The staining time for these cells, however, seems to be very critical, and variations in the results are liable to occur. The tumour cells, however, show a readiness to take up the orange *G*, the normal cells aniline blue. The surfaces of the cells described stained intensely. These results recall the differentiation of tumour cells from normal ones by their cytoplasm, unlike that of normal cells, failing to take up fluorescein-globulin stains, the reason given for this being that the protein that interacts with the globulin fraction is absent in tumour cells[5]. In the case of Mallory staining, it would seem that a protein of the scleroprotein group may be responsible for the tumour cells taking up the orange *G* dye. There remained also the possibility of the protein being tanned in some way, and that such a protein might comprise a proportion of the tumour cell surface.

Accordingly, the amelanotic Rous 1 sarcoma tissue was tested for the presence of tyrosinase, the tanning enzyme[6], and the result was positive. It may be recalled that melanotic melanomas provide a rich source of the enzyme[7], and reference has also been made to its presence in amelanotic tumour tissues[8]. Incubation of the same tissue with dihydroxyphenylalanine as the substrate resulted in melanin being intensely developed. The tumour tissue also mediated the conversion of the diphenol to melanin more readily than equivalent wet weights of normal tissues such as connective tissue from a growing cockscomb, or those of the various parts of chick embryos. Rous 1 and MH_2 tumour tissue induced by virus infection to grow on the chorio-allantoic membrane of the chick embryo also displayed an ability to mediate the conversion of the diphenol to melanin more readily than the normal membrane tissue from which the tumours were derived. These tests involved immersing the material in a saline solution of the diphenol. The process was completely blocked by phenylthiourea at $10^{-3}M$. This copper-catalyst blocking agent inhibits the activity of tyrosinase[1] without affecting cytochrome oxidase[9], which is apparently capable of catalysing the oxidation of a diphenol to melanin[7].

In the light of these observations it seemed possible that the character of tumour cells might be so coupled to copper-enzyme catalysis, that their metabolism might be more sensitive to its blockage by a chemical of specific action than that of normal cells. On this view, experiments were designed and carried out in collaboration with Dr. J. G. Carr and Mr. H. Drayton, of the British Empire Cancer Campaign Unit at the Poultry Research Centre, Edinburgh, to test the effect

Table 1. Inhibitory Effect of Phenylthiourea on the Occurrence of Rous 1 Sarcoma Metastases

		Serial No.	Primary Tumour	Metastases: Lung	Liver	Spleen	Pancreas	Heart	Mesentery	Feather follicles	Kidney	Days after inoculation: Died	Killed
Batch 3	Controls	1	+	+	+	+	+	.	.	.	.	29	
		2	+	.	.	.	.	.	.	+	.	27	
		3	+	.	.	+	+	+	.	.	.		38
		4	+	+	+	+	.	.	.	.	.		38
		5	+	+	+	+	+	+	.	+	.	36	
		6	+	+	+	.	+	.	.	.	.	36	
		7	+	+	.	.	.	.	.	.	.	37	
		8	+	+	+	.	.	+	.	.	.	31	
	Treated (1·2 mgm. daily)	1	+	+	.	.	.	.	.	.	.		38
		2	regressed	.	.	.	.	.	.	.	.		38
		3	+	.	.	.	.	.	.	.	.	30	
		4	+	.	.	.	.	.	.	+	.		38
		5	+	.	.	.	.	.	.	.	.		38
		6	regressed	.	.	.	.	.	.	.	.		38
Batch 4	Controls	1	+	+	+	.	.	.	+	.	.	26	
		2	+	+	+	.	.	.	.	.	+	19	
		3	+	+	+	.	.	.	.	.	+	20	
		4	+	+	.	.	.	+	+	.	.	21	
		5	+	.	.	.	.	.	.	.	.		26
		6	+	+	+	.	.	.	+	.	.	24	
		7	+	.	.	.	.	.	.	+	.		26
		8	+	.	.	.	.	.	.	+	.		26
		9	+	+	+	+	+	.	.	.	+	22	
		10	+	.	+	.	+	.	.	.	.	19	
	Treated (12 mgm. daily)	1	+	.	.	.	.	.	.	.	.		26
		2	regressed	.	.	.	.	.	.	.	.		26
		3	+	.	.	.	.	.	.	.	+	20	
		4	regressed	.	.	.	.	.	.	.	.		26
		5	+	.	.	.	.	.	+	.	+		26
	Treated (24 mgm. daily)	1	+	.	.	.	.	.	.	.	.		26
		2	regressed	.	.	.	.	.	.	.	.		26
		3	regressed	.	.	.	.	.	.	.	.		26
		4	regressed	.	.	.	.	.	.	.	.		26
		5	nil	.	.	.	.	.	.	.	.		26

+ = Positive for tumour

of such a chemical on the growth of Rous 1 sarcoma in tumour-bearing animals.

Six-week old Brown Leghorns inoculated in the breast and legs with the Rous 1 virus rapidly develop malignant tumours, often very large, with a high incidence of metastases established in one or several organs. The virus takes effect in 24 hr. Treatment with phenylthiourea, the compound chosen for use as the copper-catalyst blocking agent, began on the third day after inoculation. It was dissolved in saline and injected daily into four areas of the breast and legs remote from the sites of inoculation where the tumours developed.

The results shown in Table 1 illustrate the inhibitory effect of the compound on the occurrence of metastases, and how, at the higher concentrations, it affected the growth of the primary tumours.

It will already be apparent that the observations that suggested the inhibition studies do not provide an explanation for the action of the compound, which, judging from the post-mortem examinations, was selective. The treated animals certainly displayed a considerable tolerance to the compound. When it was added to a virus suspension it did not impair the tumour-inducing potency of the virus. Virus extracted from regressing tumours of treated animals also retained its ability to induce tumour growth in another host.

It seems then that the compound is exerting its effect on the cell. It is less easy, however, to suggest a rationale for the apparent selective action of the compound, because we still know practically nothing about cellular chemical organization. It has long ago been suggested, however, that the site of the depressant action of urea derivatives on the respiration of normal and tumour tissues is that part of the respiratory chain involving diphosphopyridine nucleotide, and not the oxygen terminal part[9]. This is still believed to be the case[10]. It is now thought that quinone compounds are implicated in oxidative phosphorylation[10,11], and recently a copper-enzyme mechanism has been associated with hydrogen transfer by phenol œstrogens, the diphenol derivative being oxidized by polyphenoloxidase to quinone, which is reduced by the reduced pyridine nucleotide[12]. If such a system is implicated in tissue oxidation, as seems likely, then the possibility of its blockage by phenylthiourea inhibiting cell metabolism obviously cannot be dismissed. It would be interesting to learn whether this system operates differently in the oxidation of tumour tissue. Its presence in cells, however, makes the previous suggestion of surface tanning plausible. If the non-adhesive surface property of tumour cells, upon which metastasis partly depends, were due to some form of tanning, then phenylthiourea, in blocking the process, could impair the intrinsic ability of tumour cells to invade surrounding tissue. This possibility has been discussed elsewhere[2].

It is hoped to publish with Dr. J. G. Carr and Mr. H. Drayton the details of the *in vivo* studies in due course. I am indebted to Prof. M. M. Swann and Dr. P. D. Mitchell for helpful discussion, and to Prof. A. Haddow of the Chester Beatty Research Institute for his interest in this approach to the problem of metastasis; also Dr. E. J. Ambrose for providing the hamster kidney cells.

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Immunization with Biologically Active Fractions of Deoxyribonucleic Acid and Subsequent Specific Infection of Mice

In their paper on the antigenic role of nucleic acid Lackman *et al.*[1] have shown that pneumococcal horse serum can be absorbed by homologous carbohydrate, nucleic acid 'precipitins' remaining intact. They have demonstrated in a series of absorption tests that the two 'precipitins' in question are serologically distinct. The authors suppose that they are formed in course of immunization with pneumococci.

In this laboratory the problem was investigated whether a crude fraction of deoxyribonucleic acid or possibly the purified form can be employed as a protective antigen in the case of a subsequent infection with the same micro-organism from which the crude fraction and deoxyribonucleic acid itself have been isolated.

Of seven experiments carried out on white mice, only four are to be presented here as two of them were affected by a secondary infection in the course of experiment and in the third a low infection dose was applied.

White mice (body-weight, 18–22 gm.) were immunized into their tail veins, on 3 consecutive days in a week and similarly in the following week (modified after Blix *et al.*[2]). On the seventh or eighth day after the last immunization dose the mice were infected intraperitoneally with 1,000–2,000 cells of a mucose Gram-positive culture of *Diplococcus pneumoniae*, Type II, suspended in physiological saline.

In isolating crude fractions of deoxyribonucleic acid as well as acid purified by treatment with ribonuclease with subsequent dialysis of decomposition products and in purifying deoxyribonucleic acid by removing capsule and somatic polysaccharides by calcium-alcohol fractionation the method of McCarty and Avery[4] was used. Unlike these authors, however, we used an 8-hr. plate mucose culture of Type II pneumococcus. All fractions were highly biologically active[5]. For crude fractions of deoxyribonucleic acid the amount of total nucleic acid used for immunization is presented, as revealed by a preliminary spectrophotometric estimation of optical density of the sample since immunization was carried out immediately after isolation of nucleic acid. Samples were analyzed afterwards for phosphorus[6], nitrogen by microkjeldahlization, deoxyribonucleic acid[7], ribonucleic acid[8]. Chromatographic sugar analyses[3, 9] and capillary precipitation tests involving the tested antigen (nucleic acid fraction) and anti-pneumococcal serum of type II with a high titre were also performed. Results of analyses of individual fractions with respect to their biological activity shall be published later.

The results (Table 1) show that the nucleo-proteino-polysaccharide complex, isolated from Type II pneumococcus and purified approximately to the degree of the eighth deproteinization with chloroform-octylalcohol in a total immunization dose of about 1–2 mgm. nucleic acid for one mouse given intravenously in physiological saline *p*H about 7·2 provides complete protection of white mice from a subsequent specific infection (about *LD* 3). Nucleic acid fractions lose their effectivity with further deproteinizations. After eleven deproteinizations and removal of capsular and somatic polysaccharides from a half of the isolated sample of nucleic acid (experiment 3) the presence of polysaccharides in the nucleic acid fraction does not increase the effectivity of the fraction but on the contrary it accelerates the lethal effect of a subsequent infection. In experiment 4 five different fractions after fourteen deproteinizations were used: (1) non-dialyzed fraction, containing deoxyribonucleic acid, ribonucleic acid and polysaccharides; (2) non-dialyzed precipitate (ribo- plus deoxyribo-nucleic acids), as in fraction (1), but with polysaccharides removed by calcium-alcohol fractionation; (3) non-

Table 1. IMMUNIZATION BY DEOXYRIBONUCLEIC ACID FRACTIONS ISOLATED FROM TYPE II PNEUMOCOCCUS

Exp. No.		DNA fraction: Composition of fraction	Spectrum*: min.	max.	max./min.	N/P	No. of immunised mice: i.p.	i.v.	Non imm'sd con'tl No.	Immunization dose (mgm.NA)	Infection dose No. of cells	% of death when immunised: i.p.	i.v.	% of death in non immunised control
	5	DNA+RNA+Ps**+ +P***	230	258	1·83	—	15	13		0·72		13	0	
1									15		1300			86·5
	9	idem	230	258	2·12	—	15	16		0·244		100	100	
	5	DNA+RNA+ Ps+ +P	235	258	1·66	—	4	5		1·92		25	0	
2									20		1030			75
	8	idem	230	258	2·21	—	5	6		1·92		40	0	
		DNA+RNA+ +Ps+ +P residue	230	258	2·26	—		4		1·92			50	
3	11								5		1800			80
		DNA+RNA+ +P residue	230	258	2·31	—		4		1·92			50	
		DNA+RNA+ +Ps	230	258	2·31	1·65		4		0·9			100	
		DNA+RNA	230	258	2·17	1·9		4		0·9			50	
4	14	Ps+ +DNA residue	230	258	1·97	2·1		3	20	0·3	1500		100	95
		DNA+Ps	230	258	2·78	1·61		2		0·9 (DNA)			100	
		DNA	230	258	2·3	1·82		2		0·9 (DNA)			100	

Ps = polysaccharides. P = protein. * Measured in the ultra-violet range of the SF_4 spectrophotometer (U.S.S.R.)

dialyzed supernatant (polysaccharides plus deoxyribonucleic acid), supernatant after precipitation of fraction (2) which should contain only capsular and somatic polysaccharides[4] but contained also some deoxyribonucleic acid and remained biologically active; (4) dialyzed fraction (deoxyribonucleic acid plus polysaccharides), the same as (1) but with ribonucleic acid removed by means of crystalline ribonuclease; (5) dialyzed precipitate (deoxyribonucleic acid), the same as fraction (4), but without polysaccharides. One may conclude that purified deoxyribonucleic acid or polysaccharides alone do not protect mice under the given conditions but on the contrary they seem to sensibilize them toward infection (possible immunological paralysis by pneumococcal polysaccharides[10]). It is possible that the method of isolation of deoxyribonucleic acid causes the polysaccharides of purified fraction to have no immunogenic effect.

All fractions, both crude and purified, which contained polysaccharides brought about vasodilatation after the first immunization dose, an inflammatory reaction apparent on ears and tail, accompanied by œdema or even by necrosis. Crude nucleic acid fractions caused a shock. The ratio of ribonucleic to deoxyribonucleic acid of individual fractions in a mixture of both nucleic acids amounts to 3–4 according to preliminary analyses.

It has been reported that purified deoxyribonucleic acid (up to 20 per cent protein) is not antigenic[11] and that deoxyribonucleic acid alone or with traces of bound protein is insufficient to produce transplantation immunity in homotransplantation[12]. This confirms the finding[13] that immunizing deoxyribonucleic acid from *Brucella abortus* contained 25 per cent protein along with other components.

This preliminary communication will be completed by more detailed analytical data.

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Prolonged Survival of Dystrophic Mice treated with 17α-Ethyl-19-Nortestosterone

Muscular dystrophy is an heritable disease characterized by progressive weakness and wasting of muscle, creatinuria[1], and decreased ability of muscle to take up creatine[2]. The observation that the administration of 17α-ethyl-19-nortestosterone, an anabolic agent, augments markedly the synthesis of endogenous creatine[3] led us to investigate the effect of this substance on the survival of dystrophic mice. The dystrophic mice are a strain 129 mutant which suffer from an hereditary disease resembling human muscular dystrophy in its natural history and gross and microscopic pathology[4], in the electrolyte abnormalities of residual muscle[5], and in the presence of abnormally high levels of aldolase in the serum.

Dystrophic mice obtained from the Jackson Memorial Laboratory, Bar Harbor, were divided into nine experimental groups. Aniamls given 0·5 γ/gm. body weight 17α-ethyl-19-nortestosterone (*SC*-5914) in corn oil by stomach tube three times a week starting at five weeks of age (group II) had a median survival (ST_{50}) of 22 weeks. Animals given *SC*-5914 parenterally in sesame oil (group III) had a ST_{50} of 24 weeks. In another group of animals (IV) in which the start of treatment was delayed until nine weeks of age, the ST_{50} was 18 weeks. Animals given 5·0 γ/gm. body-weight *SC*-5914 (group V) had a *ST*ss of 33 weeks. In a group of animals (VII) given 5·0 γ/gm. body-weight 17α-ethyl-Δ^4-19-norandrostenediol-3α, 17β-3-propionate (*SC*-7294) in sesame oil intramuscularly weekly the ST_{50} was 20 weeks. The ST_{50} of control mice (I) given corn oil alone by stomach tube was 13 weeks.

To rule out the possibility that prolonged survival was due to increased food intake, a group of dystrophic mice (VI) was force pair fed to group II; its ST_{50} was 15 weeks, not significantly different from that of control mice fed *ad libitum* (group I). We found water and salt retention to follow administration of these steroids to humans. Mice given 0·5 γ/gm. *SC*-7294 weekly and 2·0 γ/gm. chlorthiazide, a diuretic, twice weekly (group VIII) had a ST_{50} of 23 weeks. Another group of dystrophic mice (IX) were treated with 0·5 γ/gm. testosterone propionate in sesame oil intramuscularly three times weekly to determine whether the prolongation of life was related to androgenic activity; these mice had a ST_{50} of 15 weeks, not significantly different from that of the control dystrophic mice.

Administration of these steroids does not result in the return of any muscle function lost before the start of treatment; they seem only to retard the progression of the disease.

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Table 1. Prolongation of Survival of Dystrophic Mice

Group	Treatment	Dose	Frequency	Route	Number of Animals	ST_{50} and *S.D.* (weeks)
I	Control mice, corn oil	0·01 ml./gm.	3x/week	p.o.	16	13±2·6
II	SC-5914*	0·5 γ/gm.	3x/week	p.o.	8	22±4·6
III	SC-5914*	0·5 γ/gm.	3x/week	i.m.	6	24±4·2
IV	SC-5914*†	0·5 γ/gm.	3x/week	p.o.	7	18±5·1
V	SC-5914*	5·0 γ/gm.	3x/week	p.o.	5	33±6·1
VI	Controls force pair fed to group II	—	—	—	4	15±3·2
VII	SC-7294**	5·0 γ/gm.	once/week	i.m.	6	20±4·5
VIII	SC-7294**	5·0 γ/gm.	once/week	i.m.	4	23±3·2
	Chlorthiazide	2·0 γ/gm.	twice/week	i.m.		
IX	Testosterone propionate	0·5 γ/gm.	3x/week	i.m.	6	15±1·8

* 17α-ethyl-19-nortestosterone.
** 17α-ethyl-Δ^4-19-norandrostenediol-3α, 17β-3-propionate.
† treatment was started at 9 weeks of age. In all other groups treatment was started at 5 weeks of age.

Association. The steroids were provided by Dr. Robert L. Craig, G. D. Searle and Co., Chicago.

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ENTOMOLOGY

Secretion of Wax through the Cuticle of Insects

ON the outside of an insect there is a layer of wax or grease which is its main protection against desiccation[1]. At moulting the wax appears as an outer layer of the epicuticle about 0·1–1μ thick[2] at the time the old skin is shed. Wax may also be secreted during an instar for the repair of abrasions to the cuticle. This poses the problem of how the wax—often hard and with a m.p. above 60° C.—can be transported from the epithelium to the outside through the hydrophilic endocuticle.

Three mechanisms have been suggested. (1) The wax may pass through the pore canals; but this cannot be a general explanation for pore canals do not reach the surface in all insects. (2) The wax may be conveyed to the surface in a solvent which later evaporates, for in cockroaches there is a grease composed of a hard wax dissolved in long chain alcohols and hydrocarbons[3]. (3) The wax may be secreted in a water-soluble form in combination with a protein as it is in the eggs of ticks[4].

In most insects these hypotheses are difficult to verify because of the small quantity of wax present and the complexity of the other events taking place at moulting. However, in the last instar larva of *Calpodes ethlius* Stoll (Lepidoptera, Hesperidae) wax secretion can be observed conveniently localized in space and time.

Three days after moulting to the final instar the larva appears as in Fig. 1 (left). During the next 48 hr., a wax (m.p. 81–82° C.) is extruded as filaments from fine pores in the epicuticle over a well-defined region of the ventral surface of abdominal segments 7 and 8 (Fig. 1, right). One or two days later the larva ceases feeding and begins to pupate.

In *Calpodes* there are no pore canals from the epithelium to the epicuticle, and the surface where wax appears is separated from the cells by a hydrophilic endocuticle about 50μ thick. Sudan stains do not show any soluble protein-bound lipid in the endocuticle. The third hypothesis is more difficult to disprove but (*a*) rearing the larvæ in the presence of decane or decanol vapour does not suppress wax formation (*b*) wax continues to be secreted in an area enclosed by paraffin wax, celamel cement, arildite, rubber latex or below a glass plate sealed in place with paraffin wax, and (*c*) the wax filaments are very slightly birefringent but not crystalline.

There remains the possibility that the wax is synthesized at the surface from its constituent fatty acids and alcohols which might themselves diffuse across the endocuticle. A search was therefore made in the epicuticle for an esterase which could effect this synthesis.

Fig. 1. Last instar larvæ of *Calpodes ethlius* from the underside. Left, larva almost fully grown; right, larva about 3 days before pupation showing wax-secreting areas

An esterase is readily demonstrated with 'Tween 80', O-acetyl-5-bromoindoxyl, α-naphthyl acetate and 'Naphthol A.S.' acetate as substrates, using whole larvæ abraded to expose a cut surface of the epicuticle. 10^{-3} *M* potassium cyanide was used to inhibit the phenolase system which would otherwise produce rapid blackening. The esterase reaction is more intense over the wax secreting areas but is not confined to them, suggesting that the formation of wax filaments is but an exaggeration of normal secretion due to a greater quantity of the wax precursor. The results with O-acetyl-5-bromoindoxyl as a substrate indicate that the esterase is on the outside of the epicuticular phenolase system. In the absence of cyanide, only the deep scratches blacken due to the phenolase although an esterase is shown in the more superficial scratches by the blue deposit of indigo. There is then an esterase at the surface of the epicuticle which could synthesize wax *in situ*, the insolubility of the end product favouring the production of wax.

The observations suggest that the efficiency of insecticides containing abrasive fillers might be improved by the addition of an esterase inhibitor to prevent repair of the wax layer.

A full account will be published elsewhere.

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New Neotropical Element (*Anoplotermes*) in Indian Termite Fauna

THE termite genus *Anoplotermes* Müller[1] is, along with *Speculitermes* Wasmann[2], unique among termites in not having a soldier caste, only the workers and the reproductives being present. The two genera have a close resemblance to each other. *Speculitermes* has been regarded by certain authorities, for example, Holmgren[3] and Grassé[4], as a subgenus of *Anoplotermes*, while others[5] regard it as a full genus. We believe the latter view to be more tenable since the differences between the two genera are considerable, though they are undoubtedly closely allied. They may be keyed as follows:

1 (2). Smaller forms (head-width *c.* 0·4–1·2 mm.). Mid-dorsal spot on head either absent or greatly reduced. Antennæ shorter (1·2–1·6 times the head-width); with 14 segments; 3rd segment smaller than 2nd. Foretibia swollen. Tibial spur formula variable, either 3 : 2 : 2 or 2 : 2 : 2. Left mandible with an apical and 2 or 3 marginal teeth, the 3rd marginal, when present, very small. ..... *Anoplotermes*

2 (1). Larger forms (head-width *c.* 1·0–1·4 mm.). Mid-dorsal spot on head ('Stirnocellus' of Wasmann) large and prominent. Antennæ longer (1·5–2·0 times the head-width); with 14–15 segments; 3rd segment longer than second. Foretibia not swollen. Tibial spur formula 2 : 2 : 2. Left mandible with an apical and 2 marginal teeth. ..... *Speculitermes*

Out of 43 species of *Anoplotermes* hitherto known[5–8], 32 are neotropical, 11 Ethiopean and one nearctic (this species is also neotropical). Recently, we found a new species (to be described elsewhere) from Assam. This is the first record of the genus from the oriental region, and the discovery is of considerable zoogeographical interest.

In the closely allied genus *Speculitermes*, out of 7 known species[5,9], including two which are being described elsewhere, 4 are neotropical, 3 oriental and *none* Ethiopian.

It appears to be likely that the *Anoplotermes*–part of the *Anoplotermes–Speculitermes* complex, was evolved in the neotropical region, where it still dominates, and thence spread to the Ethiopean region, with a stray offshoot to the oriental (present account), while these land-masses were still contiguous and before they had started to drift apart according to Wegener's hypothesis[10]. The evolution of the *Speculitermes*–part is less clear since the genus is well represented in both the neotropical and the oriental regions, but in the latter case not going beyond the Indo-Malayan Subregion. Its complete absence from the intervening land-mass of Africa (Ethiopean region) is puzzling.

A fuller account will be published elsewhere.

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Probing Behaviour of *Ædes ægypti* (L.) in Response to Heat and Moisture

In studies of the host-seeking behaviour of laboratory-reared *Ædes ægypti*, response of these mosquitoes to a constant source of heat and moisture was observed. Newly emerged mosquitoes were placed in a 9 cu. ft. cage, and the numbers which probed over a convection current of warm, moist air (80–83°F.), rising into the cage through a port beneath its screen floor, were counted each day in a 16-minute observation.

All experiments were conducted at 76–78°F., 56–58 per cent relative humidity, and constant light intensity. Adult mosquitoes had access to 10 per cent sucrose solution.

In the first experiment, 100 male and 100 female mosquitoes were used, and a portion of the observer's arm was exposed at a second port beneath the cage during the observation periods. The number of females that probed from day to day over the standardized source of heat and moisture followed a rhythmic pattern, with peaks in probing activity appearing at 3-day or 4-day intervals (Fig. 1). Probing activity over the observer's arm did not follow any clearly defined pattern. A second experiment gave similar results.

Lavoipierre[1] has shown that the biting activity of fertilized *A. ægypti* females is inhibited as ovarian development proceeds from the resting stage until oviposition has occurred. In our experiments, however, ovarian development did not proceed beyond the resting stage as no blood meals were provided. Lavoipierre[2] also found an inhibition to biting following mating of virgin females with fifth stage oocytes, however, when we repeated our experiment with 100 virgin female mosquitoes, the rhythmic pattern in probing activity again appeared (Fig. 1).

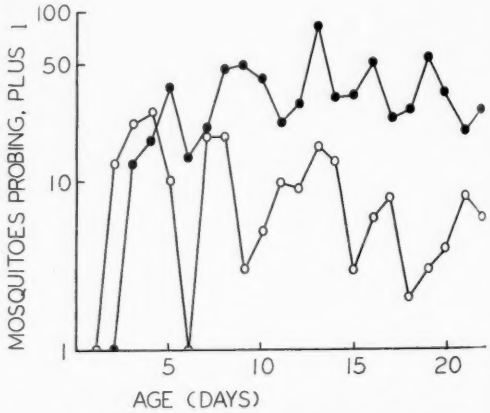

Fig. 1. Probing activity of female mosquitoes, *Ædes ægypti*, in response to heat and moisture, as shown by the total number that probed each day during a 16-minute observation. The curves are plotted on semi-logarithmic co-ordinates. ●, Curve for 100 mated females with males present; ○, curve for 100 virgin females

To determine if emanations from the body of the observer were affecting the level of probing activity, several experiments were conducted in which virgin females were shielded from human emanations by enclosing either the cage (the relative humidity now rose to 75 per cent), or the observer, in airtight plastic. Now the mosquitoes became completely non-responsive to the heat and moisture. The cage was shaken vigorously, causing most of the mosquitoes to fly, but even those flying directly through the convection current did not land and probe. Therefore in the preceding experiments, some emanation from the observer's body had conditioned the mosquitoes to probe in response to heat and moisture. In subsequent tests, it was found that the introduction of 15 to 20 c.c. of carbon dioxide into the cage caused the mosquitoes to emerge from their non-responsive state, and to probe actively over the convection current. When this procedure was repeated on a daily basis, the rhythm in probing activity again appeared.

Thus, probing by these mosquitoes in response to heat and moisture depended upon the presence of a host or upon an artificial increase in the carbon dioxide content of the air. When either of these conditions was met, the mosquitoes probed actively, with approximately a four-day rhythm in the level of probing activity. This rhythm was independent of obvious environmental factors and mating, and

was apparently not related to known processes of ovarian development. The investigations are being continued.

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MICROBIOLOGY

Comparison of Slime from Tomato and Banana Strains of *Pseudomonas solanacearum*

Pseudomonas solanacearum Smith is one of the most destructive bacterial plant pathogens in the warm regions of the world[1]. Husain and Kelman[2] have shown that highly virulent strains produce an extracellular slime which is implicated in the wilting action of the pathogen. Preliminary analysis of crude slime material isolated from culture filtrates of a tomato strain indicated it to be a polysaccharide associated with nitrogenous material.

This communication describes a preliminary examination of the slimes produced by *Pseudomonas solanacearum* strains *F2/2S* and *RE21S*, virulent to tomato and banana plants respectively. An interesting feature of these strains is the low virulence of the tomato strain towards banana plants and vice versa (Hayward, A. C., and Spence, J., unpublished work). I am trying to find whether there are differences in the composition of the slimes produced by these strains which might account for their host specificity.

The organisms were grown in 100-ml. cultures in indented 250-ml. flasks on a rotary shaker for 4 days at 26–29°C., in Husain and Kelman's liquid medium with 2 per cent sucrose. Addition of calcium carbonate (2 gm./100 ml.) to the medium stimulated growth and slime production. The cultures were centrifuged to remove cells and the slime was isolated from the supernatant fluid following Husain and Kelman's procedure. Strain *F2/2S* gave two fractions (*S*-I and *S*-II) on precipitation with 2 and 4 volumes of ethanol respectively, while strain *RE21S* gave two similar fractions with ethanol and a third one (*S*-III) with 2 volumes of acetone. When 1 per cent solutions of these fractions were tested for wilting power by immersing cuttings of tomato seedlings, all three fractions from strain *RE21S* were active, while from strain *F2/2S* only fraction *S*-II possessed wilting power. Fractions *F2/2S S*-II and *RE21S S*-III were chosen for further examination, because the high relative viscosity of their solutions suggested that these fractions contained the most polysaccharide.

Fraction *F2/2S S*-II was deproteinized by the Sevag procedure, dialysed and further fractionated by fractional precipitation. One fraction (*F2/2S S*-II*A*) was precipitated with 65 per cent ethanol; a second fraction (*F2/2S S*-II*B*) was obtained by concentrating the ethanolic supernatant liquid and pouring into a large excess of acetone. The precipitates were re-dissolved, dialysed and freeze-dried. Fraction *RE21S S*-III was deproteinized, dialysed and freeze-dried without further fractionation because of lack of material.

Preliminary experiments with paper chromatograms of acid hydrolysates sprayed with the Morgan and Elson reagents established that fractions *F2/2S S*-II, *S*-II*A*, *S*-II*B* and *RE21S S*-III all contained a hexosamine. Hydrolysis of *F2/2S S*-II*A* for 3 hr. with 2*N* sulphuric acid at 100°C. gave maximum values for hexosamine[3] and reducing sugar. All the fractions were analysed after hydrolysis under these conditions (Table 1). The sugars present in the fractions were analysed qualitatively by chromatography of concentrated neutralized hydrolysates in butanol benzene pyridine water (5:1:3:3) solvent and spraying with silver nitrate, aniline phthalate and naphthoresorcinol reagents. The following sugars (in order of decreasing concentration) were found in the fractions: *F2/2S S*-II (crude, undialysed): glucose, fructose, galactosamine, mannose (trace), unidentified sugar (sucrose was present in the unhydrolysed material). *F2/2S S*-II (dialysed): galactosamine, glucose, mannose (trace), unidentified sugar. *F2/2S S*-II*A*: galactosamine, mannose (trace). *F2/2S S*-II*B*: glucose, unidentified sugar, galactosamine. *RE21S S*-III: glucose, galactosamine, rhamnose, mannose (trace), unidentified sugar.

The unidentified sugar, present in all the fractions except *F2/2S S*-II*A*, moved slightly faster than fucose and had a *R*(rhamnose) value of 0·85–0·88 in the solvent used. It did not react with naphthoresorcinol but gave a faint pink colour with aniline phthalate and may therefore be a methylated sugar[4]; the amount was too small for further characterization. In all the fractions two further unidentified components (*R*(rhamnose) 0·80 and 1·10) were detected as pink spots on chromatograms sprayed with silver nitrate; they did not appear with the other reagents and are not thought to be sugars. The hexosamine in fraction *F2/2S S*-II*A* was confirmed to be galactosamine by isolation on a column of 'Zeokarb' 225 resin, and the detection of lyxose after treatment with ninhydrin[5]. Hydrolysis of this fraction with *N* acetic acid released a maximum value of 22 per cent N-acetylgalactosamine[6] after 12–18 hr.

Table 1

Fraction	Total reducing sugar (per cent)	Galactosamine (per cent)	Total nitrogen (per cent)	Total N-galactosamine nitrogen (per cent)	Protein (per cent)
F2/2S S-II crude	23·3	13·0	3·80	2·8	17·5
S-II*A*	35·7	35·7	6·60	3·8	23·7
S-II*B*	15·6	9·2	8·95	8·2	51·2
RE21S S-III	33·4	28·0	6·64	4·6	28·1

The fractions contained more nitrogen than could be accounted for as hexosamine (Table 1). The difference was calculated as protein; examination of the ultra-violet spectra showed the absence of nucleic acid. To identify the amino-acids, samples were hydrolysed with 6*N* hydrochloric acid for 24 hr. at 100°C. and dried several times *in vacuo* over solid sodium hydroxide, and then examined chromatographically, using butanol acetic acid water (4:1:5) solvent and a ninhydrin-copper nitrate spray[7]. The following amino-acids were found in all the fractions in the same relative amounts: lysine, glycine, glutamic acid, alanine and hydroxyproline (trace). In addition, all the fractions were found to contain an unidentified amino-acid which moved at the same rate as histidine but which gave a different colour with the spray; it was shown to be neither arginine, glutamine nor asparagine.

These results suggest that the extracellular slimes of these pathogenic strains of *Pseudomonas solanacearum* consist of mixtures of polysaccharides and a relatively simple peptide. The only qualitative difference between the fractions of the two strains examined here was the presence of rhamnose in the slime of strain *RE21S*, which was absent in that of strain *F2/2S*. Quantitative study of unfractionated slime from these strains, followed by complete separation and analysis of the components, are necessary for full characterization of the slimes;

but the present preliminary results suggest that the differences found in the slimes of these strains, as represented by the fractions analysed, are not sufficient to account for the host specificity of these plant pathogens.

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EUGENICS

Urinary Excretion of β-Amino-*iso*butyric Acid in Mongolism

Various studies have suggested that the excretion of β-amino-*iso*butyric acid in human urine is under genetic control[1-4]. This amino-acid is derived from the metabolism of thymine via dihydrothymine and β-ureido-*iso*butyric acid[5]. Variation in excretion of β-amino-*iso*butyric acid may be controlled by a single gene pair, with high excretors being homozygous for a single recessive gene and low excretors being either heterozygous or homozygous for the dominant allele[6]. It also has been suggested that low and high excretors may differ in their metabolism of β-amino-*iso*butyric acid[7]. An increased incidence of high excretors has been found in certain ethnic groups, notably the Athabascan and Apache Indians of North America, the Black Caribs of British Honduras, and the Chinese and Japanese[4,6,8]. High excretors of β-amino-*iso*butyric acid are relatively uncommon in populations of Caucasian origin[1,2,3].

Wright and Fink[9] have reported an increased incidence of high excretors of β-amino-*iso*butyric acid in children who have mongolism (43 per cent) compared with normal children (7 per cent) and children who have unclassified mental deficiency (17 per cent). A proof of this finding was desired. A 47th chromosome has been observed in the cells of mongols[10,11], and this extra chromosome may have biochemical effects.

In the present study, urine specimens were collected from 51 mongoloid children, 57 non-mongoloid mental defectives, and 61 normal children and were frozen until used. The creatinine concentration of each specimen was determined by a modification of the Jaffe reaction. Aliquots of urine containing 25 μgm. of creatinine were chromatographed two-dimensionally on Whatman No. 1 paper in pyridine/acetone/3 *N* ammonia (45:30:25) followed by *iso*propyl alcohol/formic acid/water (8:1:1). The chromatograms were sprayed with 0·2 per cent ninhydrin in 95 per cent ethanol, dried, heated at 80°C. for 5 min., and finally were sprayed with 0·25 *N* nickel sulphate solution. With this procedure, as little as 0·25 μgm. of β-amino-*iso*butyric acid can be detected readily. When β-amino-*iso*butyric acid was detected in a urine specimen, the amount was determined on paper chromatograms by visual comparison of spots obtained from appropriate aliquots of urine with those obtained from graded amounts of the authentic compound. Chromatography was carried out in a special 'smog'-free room, in order to avoid the destructive effects of oxidants present in the Los Angeles atmosphere.

Table 1. Urinary excretion of β-amino-*iso*butyric acid by mongoloid and normal children and by non-mongoloid defectives

β-Amino-*iso*butyric acid μgm./mgm. creatinine	Number of Subjects		
	Mongoloid	Normal	Non-mongoloid defectives
Less than 70 (0–66)	49	57	54
More than 70 (88–288)	2	4	3
Total	51	61	57

The data on urinary excretion of β-amino-*iso*butyric acid are presented in Table 1. Only 4 per cent of the mongols, 5 per cent of the non-mongoloid mental defectives, and 7 per cent of the normals are high excretors; that is, individuals excreting more than 70 μgm. of β-amino-*iso*butyric acid per mgm. of creatinine. The range of 70–200 μgm. per mgm. of creatinine was suggested by Harris[1] for high excretors following an examination of 345 subjects, and also was used by Calchi-Novati *et al.*[2] with 792 subjects. A distinct high excretor group was not observed by Gartler in one study[3]: more recently, however, Allison, Blumberg, and Gartler[6] have presented results which appear to confirm the range selected by Harris[1] for high excretors. The low percentage of high excretors found among mongols in the present investigation is comparable to that reported by others[1,2,3] for normal individuals of Caucasian origin.

The excretion of β-amino-*iso*butyric acid was expressed by Wright and Fink[9] in terms of urine volume. The significance and reproducibility of this parameter are questionable because the dilution of different urine specimens can vary greatly; it is more reliable to use a timed collection period or volumes containing equivalent amounts of creatinine. The present results, nonetheless, were recalculated on the same basis for the purpose of comparison: 6 per cent of the mongols, 5 per cent of the non-mongoloid defectives, and 10 per cent of the normal subjects excreted 0·5 to 9·0 millimoles of β-amino-*iso*butyric acid per litre of urine, which was the range designated by Wright and Fink[9] for high excretors. The difference in the percentage of high excretors in the three groups of subjects is not significant, regardless of the manner of computation, and it is concluded that the incidence of high excretors is not increased in mongolism.

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